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REPORT

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MAMMALIAN TOXICITY OF MUNITIONS COMPOUNDS
PHASE II: EFFECTS OF MULTIPLE DOSES
PART III: 2,6-DINITROTOLUENE

PROGRESS REPORT NO. 4
July 1976

Contract No. DAMD-17-74-C-4073
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For

Project Officer: Dr. Jack C. Dacre
Environmental Protection Research Division
U.S. Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, Frederick, Maryland 21701

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PHASE II: Effects of Multiple Doses

PART III: 2,6-Dinitrotoluene

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by

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U.S. Army Medical Research and Development Command
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The effects of oral doses of 2,6-dinitrotoluene (2,6-DNT) after administration to dogs, rats and mice for up to 13 weeks were studied. The effects of 2,6-DNT on drug metabolizing enzymes in rats were also studied.

In dogs, 4 mg/kg/day was slightly toxic; 20 mg/kg/day was very toxic and lethal to some; 100 mg/kg/day was lethal to all within 8 weeks. In rats, 7 mg/kg/day had no effects, 35 (males) or 37 (females) mg/kg/day and 145 (males) or 155 (females) mg/kg/day had dose-related toxicity. In mice, 11 mg/kg/day had

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no effect, while 51 (males) or 55 (females) mg/kg/day and 289 (males) or 299 (females) mg/kg/day had dose-related toxicity. Target organs included the blood (methemoglobin and sequelae), testes (depressed spermatogenesis), liver (degeneration, bile duct hyperplasia), neuromuscular system (incoordination and rigid paralysis) and kidney (degeneration).

Toxic doses of 2,6-DNT in rats increased drug-metabolizing enzyme activity in rat liver.

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PREFACE

This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110, under U.S. Department of the Army Contract No. DAMD-17-74-C-4073, MRI Project No. 3900-B, "Munition Compounds Mammalian Toxicity Study." The work was supported by the U.S. Army Medical Bioengineering Research and Development Laboratory, USAMRDC, Department of the Army. Cpt. John P. Glennon, Dr. Jack C. Dacre, Dr. David H. Rosenblatt and Cpt. Robert Rice, Environmental Protection Research Division, USAMBRDL, are the consecutive technical monitors for the project.

This work was conducted in the Biological Sciences Division, under the direction of Dr. William B. House, between 15 November 1974 and 30 April 1976. The experimental work was directed by Dr. Cheng-Chun Lee, Assistant Director, Biological Sciences Division for Pharmacology and Toxicology with the assistance of Dr. Harry V. Ellis, III, Associate Pharmacologist, and Mr. John J. Kowalski, Assistant Biologist. Dr. John R. Hodgson, Senior Biochemist, supervised the studies on metabolism, cytogenesis and mutagenesis. Dr. Robert D. Short, Associate Pharmacologist, supervised the in vivo study on drug metabolizing enzymes. Dr. J. C. Bhandari, Associate Veterinary Pathologist, supervised the necropsy and the histology preparation and performed the microscopic examination. Mr. Thomas W. Reddig (ASCP certified M.T.), Laboratory Supervisor, supervised the hematology and clinical laboratory tests. Mr. Jan L. Minor, Assistant Toxicologist, supervised the computer program and analysis of experimental data. Technical personnel included Bruce S. Andersen, Mary A. Kowalski, Ellen R. Ellis, Ernesto A. Castillo, Judith D. Girvin, Patricia L. Wilkerson, Bhanu S. Gosalia, Laurel M. Halfpap and William M. Bracken.

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MAMMALIAN TOXICITY OF MUNITION COMPOUNDS
PHASE II: Effects of Multiple Doses
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EXECUTIVE SUMMARY

The effects of 2,6-DNT after oral administration for up to 13 weeks were investigated in dogs, rats and mice. The effects of 2,6-DNT on drug metabolizing enzymes in rats were also studied.

In dogs, oral administration of 4 mg/kg/day had minimal toxic effects with mild splenic hematopoiesis in some dogs. Doses of 20 or 100 mg/kg/day were more toxic, producing decreased feed intake leading to weight loss, listlessness, incoordination leading to rigid paralysis with occasional tremors, methemoglobinemia leading to Heinz bodies and anemia with compensatory reticulocytosis and extramedullary hematopoiesis, lymphoid depression leading to peripheral lymphocytopenia, bile duct hyperplasia, degenerative and inflammatory changes in the liver and kidney with elevated serum alkaline phosphatase, SGPT, and/or BUN, and degeneration and atrophy of the spermatogenic cells of the testes. The effects in dogs given 100 mg/kg/day were more numerous and more severe and appeared earlier than in dogs given 20 mg/kg/day. All dogs given 100 mg/kg/day died between the second and the 8th week; only two dogs given 20 mg/kg/day died, both in the 9th week. There was great variation in the onset of symptoms among dogs given the same dose. The effects of 2,6-DNT were partially reversed in 4 weeks and completely reversed in 19 weeks after the cessation of treatment. Treatment with 2,6-DNT did not affect serum immunoglobin E titers.

In rats, 2,6-DNT intake of the males fed the low, middle or high level of 2,6-DNT in the feed averaged 7, 35 and 145 mg/kg/day, respectively. The 2,6-DNT intake of the females averaged 7, 37 and 155 mg/kg/day, respectively. The low level produced no unequivocal effects. The middle level decreased feed consumption and weight gain and increased SGPT in some rats. There were extramedullary hematopoiesis in the spleen and/or liver, bile duct hyperplasia, and/or depression of spermatogenesis and testicular atrophy. The high level also produced methemoglobinemia, Heinz bodies, anemia and compensatory reticulocytosis. The effects in high dosage rats were more severe and occurred earlier. As for dogs, there was partial recovery in rats 4 weeks after the treatment was discontinued. The middle level of 2,6-DNT in the feed (about 35 mg/kg/day) for 6 weeks increased the number of chromatid breaks in lymphocyte culture. Continuing the feeding for 13 weeks also caused an increase in tetraploids in kidney cultures. 2,6-DNT was not mutagenic in the specific locus mutation test

using Chinese hamster ovary cultures and did not affect the serum immunoglobin E titers.

In mice, 2,6-DNT intake of the males fed the low, middle or high level of 2,6-DNT in the feed averaged 11, 51 and 289 mg/kg/day, respectively. The females consumed an average of 11, 55 and 299 mg/kg/day, respectively. The low level produced no effects. The middle or the high level produced decreases in feed consumption and weight gain, extramedullary hematopoiesis, depression of spermatogenesis and atrophy in the testes, bile duct hyperplasia, and death. Mice fed the high level were more severely affected than those fed the middle level. As for dogs and rats, there was partial recovery in mice 4 weeks after cessation of treatment.

The high level of 2,6-DNT in the feed (0.25%) for 4 weeks decreased zoxazolamine paralysis time and increased hepatic nitroanisole O-demethylase activity in rats, indicating an increase in drug-metabolizing enzyme activity in the liver.

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MAMMALIAN TOXICITY OF MUNITION COMPOUNDS
PHASE II: Effects of Multiple Doses
PART III: 2,6-Dinitrotoluene
(Report No. 4)

INTRODUCTION

Under Contract No. DAMD-17-74-C-4073, entitled "Munition Compounds Mammalian Toxicity Study," we conducted Phase I studies on the effects of acute exposure of various munition compounds.[1] During Phase II, we studied the effects of multiple exposure to selected compounds including trinitroglycerin (TNG) 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT) and nitrocellulose (NC). This report summarizes the results of Phase II studies on 2,6-DNT. Subacute and subchronic toxicities were performed in dogs, rats and mice to determine the maximum tolerated dose and to define the biological nature and target organ(s) of the toxic effects. Reversibility of any adverse effects was determined. Mutagenicity of the compound was assessed. Immunologic response was studied by the detection of the serum IgE antibodies. Effects of the test compound on drug metabolizing enzymes were investigated in in vivo and in vitro studies.

I. DOGS

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I. DOGS

A. Subacute and Subchronic Toxicities and Reversibility

1. Introduction

These studies were performed to define the nature and extent of effects of 2,6-DNT on the biological system at the biochemical and cellular levels and to elucidate the dose-response relationship in dogs after administration for 4 weeks and 13 weeks. The reversibility of any adverse effects was studied after the treatment of 2,6-DNT was discontinued for 4 weeks.

2. Material and Methods

a. Number of Dogs, Sex and Treatment

A total of 32 young healthy beagle dogs (Hazelton Research Animals, Cumberland, VA), weighing between 6.0 and 12.2 kg, were used for these experiments. The dogs were acclimated and observed carefully in our animal quarters for 3 weeks after their arrival from the supplier. They were divided into four groups, each consisting of four males and four females. The average weights of all groups were kept close.

After acclimation, the dogs were trained to eat the food at the same time each day. Each dog was placed in a metabolism cage and given a measured amount of Purina dog chow. The dog was returned to his pen 30 minutes later and the uneaten feed estimated. Training continued until consumption was stable. Water was available ad libitum in the metabolism cages and pens.

Three groups of dogs were given 4, 20 or 100 mg/kg/day of 2,6-DNT in capsules. 2,6-DNT, obtained from K and K Laboratories (Cleveland, OH), was diluted with lactose USP and weighed into hard gelatin capsules. Each dog received one capsule daily. Capsules were prepared weekly, and the amount of 2,4-DNT adjusted accordingly. The fourth group served as the controls and was given lactose-filled capsules daily throughout the experiments.

b. Experimental Procedures

All dogs were observed daily for behavioral changes and toxic signs. Body weights of all dogs were recorded weekly. Feed consumption was recorded daily. Blood samples were collected for laboratory tests three times before treatment at weekly intervals and at 4, 8, 13 and/or 17 weeks during experiment. The tests included hematology, clinical blood chemistry

tests, and serum electrolytes. For fasting blood glucose, the dogs were bled before their daily feeding. At termination or when moribund, the dogs were euthanized with an overdose of sodium pentobarbital and examined for gross lesions. Weights of liver, spleen, kidneys, brain, adrenals, thyroid and gonads were recorded. Relative organ weights were calculated. Various tissues were removed, fixed, processed, sectioned and stained for microscopic examination of lesions. The procedures for hematology, clinical blood chemistry tests and histopathology, and the normal values are given in Appendix I.

The concentrations of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} in serum were determined with the atomic absorption spectrophotometer, according to the procedure used by Pybus,[2] originally described for Ca and Mg. The resonance lines used for the analysis of each of the elements are: Ca, 4227 Å; Mg, 2852 Å; Na, 3302 Å; and K, 7665 Å. Sodium was determined by using the 3302 Å line rather than the more sensitive 5890 Å line to avoid large dilutions of the serum. In this way, Ca, Mg, and Na were determined after a 50-fold dilution (0.2 mg serum to 10 ml) of the serum with 1,000 ppm strontium in 0.1 N perchloric acid. Potassium was determined after a second dilution (1:1) or a total of 100-fold dilution. Phosphate interference and the interference of sodium on potassium were eliminated by the addition of strontium. The perchloric acid was used to remove protein interference. The serum chloride concentration was determined with a Buchler-Cotlove chloridometer.

Bromosulfophthalein (BSP) retention test was performed at termination. A single dose of 5 mg/kg of the sterile test dye (Dade, Miami, FL) was injected intravenously following fasting for 16 hours. Serum level of the dye at 15 minutes was determined and the percent of retention in the plasma was calculated.[3]

The results of the various parameters were compared with the respective baseline levels and/or with those of the control groups at the respective time interval according to the Dunnett's multiple comparison procedure.[4]

c. Experimental Design

At the end of 4 and again at 13 weeks of continuous treatment, one male and one female dog from each group were euthanized for necropsy. The treatment for one other male and female dog from each group was discontinued at the end of 4 and 13 weeks and they were euthanized at the end of 8 and 17 weeks, respectively, to study the reversibility of any adverse effects. Because of the severity of symptoms observed, the dogs receiving the high dose placed on the reversibility study after 4 weeks were continued for 19 weeks (23 weeks total) before they were euthanized.

3. Results

a. General Observations, Body Weight and Feed Intake

At the start of the second week, two of the dogs (Nos. 25 and 29) receiving the high dose (100 mg/kg/day) of 2,6-DNT appeared listless with pale gums. These and similar symptoms were soon noted in the other dogs receiving the high dose and developed into a characteristic syndrome. The milder symptoms seen were listlessness, incoordination, lack of balance, pale gums, dark urine and weakness, particularly of the hind limbs. Purposive movement became difficult. As the symptoms worsened, muscles became stiff, until the dogs were unable to move. Occasional brief tremors were noted, but they could not be characterized as large-scale fasciculations, clonus, shivering or any other ordered sign. Terminal signs noted in some dogs included yellow gums and darkened sclera. Intensity of symptoms was generally minimal at the time of dosing and most intense 1 to 2 hours later.

Since these dogs were eating little during week 3, they were given canned dog feed instead of dry dog feed and ate somewhat more. One dog died during the second week, and the last dog on this treatment died in the 8th week.

Dogs receiving the middle dose (20 mg/kg/day) showed similar symptoms, but in milder form. Symptoms were not seen until week 4. The dogs were not switched to canned feed until week 8. The only unscheduled deaths were two females (Nos. 18 and 20) in week 9. The other dogs often had a lessening of toxic symptoms in the last weeks of the study. During the reversibility studies, symptoms decreased rapidly.

Throughout the study, the control and the dogs receiving the low dose (4 mg/kg/day) showed no remarkable symptoms.

Body weights of these dogs before, during and after treatment are summarized in Table 1. Control dogs and the dogs receiving the low dose either gained weight or had minor fluctuations. The middle dosage dogs showed no weight change until week 4 or 5, when they began to lose weight. The loss correlated with the toxicity syndrome described above. The two dogs who died lost about 25% of their body weight, but some survivors gained weight. The high dosage dogs lost weight from the first week of treatment. Some dogs lost over 25% of their initial body weight before they died. The dogs who died at later times showed less toxic signs but lost more weight.

Feed consumption, shown in Tables 2 and 3, correlated well with weight changes. From the beginning of dosing, the dogs given the high dose ate about half as much as the controls. The middle dosage dogs ate

somewhat less than the controls, especially after week 5. The low dosage dogs ate amounts comparable to control dogs. When the severely affected dogs were placed on the canned dog feed instead of the usual dry feed, they ate somewhat more. During the reversibility studies, the affected dogs quickly returned to normal feed consumption rates. There was considerable variation in consumption day to day. However, the weekly averages were fairly consistent.

b. Blood Analyses

Because one high dosage dog died during week 2 and two more were severely affected, blood from all surviving dogs was analyzed at the end of week 2, as an addition to the scheduled analyses. The results of the laboratory data from the control dogs are shown in Table 4. The high percentage of methemoglobin at 8 weeks is unexplained, probably an artifact. Various peripheral blood elements, clinical blood chemistry and serum electrolytes of these dogs fluctuated during the experiment. The changes were mild and of no clinical importance.

The results of the laboratory data from the dogs receiving 4 mg/kg/day of 2,6-DNT (Table 5) were similar to those of the control dogs. Some small fluctuations were of no toxicological significance.

Administration of 20 mg/kg/day of 2,6-DNT caused a number of significant effects in 2 weeks (Table 6). There was anemia with decreased hematocrit, hemoglobin concentration and a compensatory reticulocytosis. The size of erythrocytes increased. Small amounts of methemoglobin were seen at 8 weeks and Heinz bodies at 13 weeks of treatment. SGPT was increased at 8 and 13 weeks. Fluctuations in platelet and leukocyte counts, clotting time and BUN are within normal limits. Female dog No. 20 was moribund in week 9. She was severely anemic with large amounts of Heinz bodies and methemoglobin. Both transaminases and alkaline phosphatase were elevated.

Dogs receiving 100 mg/kg/day of 2,6-DNT were more severely affected (Table 7). After 2 weeks, the erythrocyte count was only one-third of the baseline, with one-sixth of the cells being reticulocytes and one-fifth nucleated. Erythrocyte volume and cell hemoglobin were increased, but cell hemoglobin concentration was decreased, reflecting the immaturity of many of the cells. Other changes included leukocytosis with increased percentage in neutrophils and decreased percentage in lymphocytes and increases in immature leukocytes (bands), alkaline phosphatase and SGPT in some dogs. In addition, there were changes in serum electrolytes. After 4 weeks, the effects were similar. SGPT, alkaline phosphatase and serum electrolytes had returned to normal. Three dogs (Nos. 32, 31 and 28) when moribund between 5th and 8th weeks also had Heinz bodies, two dogs had elevated BUN and one dog had methemoglobinemia and elevated SGPT. The high blood glucose suggests non-fasting state.

Laboratory data from dogs allowed to recover without 2,6-DNT for 4 weeks or longer are shown in Tables 8, 9 and 10. The dogs given 20 mg/kg/day for 4 or 13 weeks recovered after 4 weeks (Tables 8 and 10). The dogs given 100 mg/kg/day for 4 weeks did not recover after 4 and 9 weeks, but were fully recovered after 19 weeks (Tables 8 and 9).

BSP retention was not affected by the 2,6-DNT treatment (Table 10).

c. Organ Weights

The absolute and relative organ weights of dogs terminated at various times are shown in Tables 11 through 14, respectively. The spleens of dogs given 2,6-DNT for 4 or 13 weeks were enlarged, and the testes of the dogs given 2,6-DNT for 13 weeks were decreased. Organ weights of dogs given middle or low dose were not apparently altered.

d. Gross and Microscopic Examination of Tissues

The dogs given 100 mg/kg/day of 2,6-DNT were in poor nutritional condition with little body fat. They were dehydrated and jaundiced. The two dogs (Nos. 18 and 20) receiving 20 mg/kg/day who died in week 9 were similarly emaciated and jaundiced. The other dogs appeared in good condition. Tissue lesions seen in dogs autopsied at various times are listed in Tables 15 through 18, respectively.

Control dogs and dogs receiving 4 mg/kg/day for 4 weeks (Table 15) had a few spontaneous lesions, including inflammation in the lung or tonsil, and an increase in parafollicular cells in the thyroid. The female given 4 mg/kg/day (No. 16) had several graafian follicles, but no corpora lutea. This dog also had mild extramedullary hematopoiesis in the spleen, characterized by hemosiderosis, nucleated erythrocytes and granulocytic cells. Since this was seen in dogs given this dose for 13 weeks (Table 16), and in dogs given higher doses, it was considered to be an effect of the 2,6-DNT. Dogs given 20 mg/kg/day or 100 mg/kg/day had spontaneous lesions in the lung, ovary, thyroid and tonsil similar to those seen in the control and low dosage dogs. In addition, 20 or 100 mg/kg/day of 2,6-DNT caused extramedullary hematopoiesis in the liver and spleen, bile duct hyperplasia, degeneration and/or subacute inflammation in the liver, and degeneration and/or depression of spermatogenesis in the testes. The incidence and severity of these lesions were generally proportional to the dose. Furthermore, 100 mg/kg/day also caused lymphoid depletion in the spleen and lymph node, and involution of the thymus.

After treatment for 13 weeks, 20 or 100 mg/kg/day of 2,6-DNT caused similar lesions in the liver and spleen (Table 16). In addition, there were dilated tubules, foci of inflammation, degeneration, yellow pigment and/or casts in tubules of the kidney. The high dose also caused

lesions in the testes, lymph node and thymus. The lesions were usually more numerous and more severe than those seen in dogs treated for 4 weeks. There were also extramedullary erythropoiesis and lymphoid depletion in the spleen of dogs given the low dose (4 mg/kg/day). A variety of other lesions in the control dogs and dogs given various doses of 2,6-DNT were inconsistent and spontaneous lesions, not related to the compound.

The dogs treated for 4 weeks and then allowed to recover showed some improvement in 4 weeks, with lesser amounts of extramedullary hematopoiesis and testicular toxicity (Table 17). The two dogs given 100 mg/kg/day and allowed to recover for 19 weeks showed complete recovery. Dogs treated for 13 weeks did not show full recovery (Table 18). Female dog No. 10, given 4 mg/kg/day, still had minimal bile duct hyperplasia from the treatment. Male dog No. 17, given 20 mg/kg/day, still had various lesions in the liver, kidney and testes.

4. Discussion and Conclusions

In dogs, oral administration of 4 mg/kg/day of 2,6-DNT had minimal toxic effects with mild splenic hematopoiesis in some dogs. Larger doses were more toxic, some dogs dying within 9 weeks of treatment with 20 mg/kg/day and all dogs dying before the completion of 8 weeks of 100 mg/kg/day. Toxic effects included decreased feed intake leading to weight loss, listlessness, incoordination leading to rigid paralysis with occasional tremors, methemoglobinemia leading to Heinz bodies and anemia with compensatory reticulocytosis and extramedullary hematopoiesis, lymphoid depression leading to peripheral lymphocytopenia, bile duct hyperplasia and degeneration and atrophy of the spermatogenic cells of the testes. In addition, there were degenerative and inflammatory changes in the liver and kidney leading to elevated serum alkaline phosphatase, SGPT and/or BUN in some dogs. In general, effects in dogs given 100 mg/kg/day were more numerous and severe than those seen in the lower doses, and lesions in dogs treated for 13 weeks were more numerous and severe than those treated for 4 weeks. There were great variations in individual susceptibility. When high dosage male No. 25 died on day 12, the other high dosage dogs had minimal apparent effects. Similarly, two middle dosage females (Nos. 18 and 20) died in week 9 after losing considerable body weight, whereas two males receiving the same dose (Nos. 17 and 19) survived 13 weeks of study with little weight loss or slight weight gain.

A decreased serum calcium concentration can produce tetany,[5/] which resembles the neuromuscular signs seen. However, this requires hypocalcemia of 3.5 meq/liter or less, and the neuromuscular effects in these dogs were not due to hypocalcemia. The lowest serum calcium concentration in these dogs was 4.2 meq/liter, and most values were within the normal range of 4.5 to 5.5 meq/liter. However, an interaction between

2,6-DNT or a metabolite and calcium near the motor end plate might produce a local hypocalcemia. Such a mechanism is purely speculative.

Qualitatively and quantitavely, most of the effects of 2,6-DNT were similar to those previously reported from 2,4-DNT.[6] However, with 2,4-DNT, extramedullary hematopoietic activity was not evident. This may reflect the ability of the dog to adapt to a given dose of 2,6-DNT but not 2,4-DNT, or due to individual variation. Some kidney lesions were seen with 2,6-DNT only, and some central nervous system (CNS) lesions with 2,4-DNT only. All of these lesions were relatively mild. The hypocalcemia in dogs treated with 2,4-DNT was not sufficient to induce tetany. Similar neuromuscular effects were observed in dogs given either compounds, but the mechanism is unexplained.

B. Immunologic Response to 2,6-DNT

1. Introduction

In humans, anaphylactic reactions were associated with a high immunoglobin E (IgE) titer.[7] IgE, the allergic or hypersensitive antibody of dogs treated with 2,6-DNT, was determined.

2. Material and Methods

The immunodiffusion technique of Mancini et al.[8] was used for determination of serum IgE titer. Replicate 1-ml samples of serum from the control dogs and dogs treated with various doses of 2,6-DNT at various intervals were placed in wells in an immunodiffusion chamber along with suitable standards. These dogs were used for subacute and subchronic toxicity studies as described in Section I.A. The diffusion chamber was incubated at 37°C for 48 hours and the diameter of the precipitin ring was measured. Since the square root of the diameter is directly proportional to the concentration of the antibody, the IgE concentration was quantitated with the standard antibody reagent.

3. Results and Conclusion

The results of IgE concentration of control dogs and dogs treated with 2,6-DNT are summarized in Table 19. Treatment of various doses of 2,6-DNT for 0, 2, 4 and 13 weeks did not cause any apparent changes on serum concentration of IgE.

C. Summary

Oral administration of 4 mg/kg/day had minimal toxic effects with mild splenic hematopoiesis in some dogs. Doses of 20 or 100 mg/kg/day were more toxic, producing decreased feed intake leading to weight loss, listlessness, incoordination leading to rigid paralysis with occasional tremors, methemoglobinemia leading to Heinz bodies and anemia with compensatory reticulocytosis and extramedullary hematopoiesis, lymphoid depression leading to peripheral lymphocytopenia, bile duct hyperplasia, degenerative and inflammatory changes in the liver and kidney with elevated serum alkaline phosphatase, SGPT and/or BUN, and degeneration and atrophy of the spermatogenic cells of the testes. The effects in dogs given 100 mg/kg/day were more numerous and more severe and appeared earlier than in dogs given 20 mg/kg/day. All dogs given 100 mg/kg/day died between the second and the 8th week; only two dogs given 20 mg/kg/day died, both in the 9th week. There was great variation in the onset of symptoms among dogs given the same dose. The effects of 2,6-DNT were partially reversed in 4 weeks and completely reversed in 19 weeks after the cessation of treatment. Treatment with 2,6-DNT did not affect serum immunoglobin E titers.

TABLE 1

BODY WEIGHTS OF DOGS TREATED WITH 2,6-DNT IN CAPSULES

Dose (mg/kg/day)	Dog No.	Sex	Body Weights (kg) Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	7	M	11.5	12.4			
0	8	F	6.8	7.8			
4	15	M	12.0	11.9			
4	16	F	7.0	8.3			
20	23	M	11.0	10.3			
20	24	F	7.9	8.9			
100	25	M	9.0	7.2[a]			
100	26	F	7.4	5.6			
0	5	M	8.6	8.4[b]	8.3		
0	6	F	7.0	9.0[b]	8.5		
4	13	M	10.0	10.4[b]	9.8		
4	14	F	6.8	7.8[b]	7.9		
20	21	M	9.2	9.3[b]	9.3		
20	22	F	7.6	8.5[b]	8.7		
0	3	M	9.9	9.6	9.1	11.4	
0	4	F	6.0	6.9	6.5	8.0	
4	11	M	9.2	10.0	9.4	12.0	
4	12	F	7.0	8.4	7.5	9.6	
20	18	F	7.9	7.8	6.3	6.0[c]	
20	19	M	9.0	9.4	8.9	11.4	
20	20	F	7.0	7.3	6.0	5.2[c]	
100	32	F	7.0	5.5	4.9[d]		
100	27	M	7.4	5.8	6.0[e]		
100	28	F	7.0	6.2	6.6[f]		
100	31	M	12.0	8.8	6.7[f]		
0	1	M	10.0	10.2	10.3	11.8[b]	11.8
0	2	F	8.0	9.5	8.7	10.4[b]	9.2
4	9	M	10.5	10.8	11.0	12.6[b]	12.0
4	10	F	8.9	10.3	10.4	12.4[b]	10.8
20	17	M	10.0	10.0	8.9	9.2[b]	8.6
100	29	M	11.0	8.9[b]	9.8	12.4	11.8
100	30	F	7.6	6.8[b]	7.2	9.0	8.0

a/ Terminal weight before necropsy during week 2.
b/ Dosing discontinued thereafter.
c/ Terminal weight before necropsy during week 9.
d/ Terminal weight before necropsy during week 6.
e/ Terminal weight before necropsy during week 7
f/ Terminal weight before necropsy during week 8.

TABLE 2

FEED CONSUMPTION OF DOGS TREATED WITH 2,6-DNT

Dose (mg/kg/day)	Dog No.	Sex	Feed Consumption (gm/day) Week 4	Week 8	Week 13	Week 17
0	7	M	760			
0	8	F	670			
4	15	M	760			
4	16	F	520			
20	23	M	690			
20	24	F	640			
100	25	M	380[a]			
100	26	F	330			
0	5	M	720[b]	640		
0	6	F	680[b]	620		
4	13	M	750[b]	0		
4	14	F	550[b]	480		
20	21	M	620[b]	650		
20	22	F	680[b]	640		
0	3	M	690	450	540	
0	4	F	240	500	700	
4	11	M	760	760	640	
4	12	F	670	580	520	
20	18	F	490	240	70[c]	
20	19	M	680	560	450	
100	20	F	490	300	100[c]	
100	32	F	190	350[d]		
100	27	M	270	260[e]		
100	28	F	370	220[f]		
100	31	M	350	160[f]		
0	1	M	760	450	540[b]	610
0	2	F	610	520	500[b]	580
4	9	M	760	680	620[b]	680
4	10	F	680	760	580[b]	750
20	17	M	670	460	340[b]	540
100	29	M	490[b]	760	690	610
100	30	F	300[b]	520	580	610

a/ Moribund during week 2; data for last week alive.
b/ Dosing discontinued thereafter.
c/ Moribund during week 9; data for last week alive.
d/ Moribund during week 6; data for last week alive.
e/ Moribund during week 7; data for last week alive.
f/ Moribund during week 8; data for last week alive.

TABLE 3

AVERAGE FEED CONSUMPTION OF DOGS
TREATED WITH 2,6-DNT

2,6-DNT (mg/kg/day)	Feed Consumed gm/dog/day		gm/kg/day	
	Male	Female	Male	Female
0	599 ± 27[a/]	612 ± 25	60.1 ± 3.0	78.0 ± 4.2
4	646 ± 22	608 ± 16	61.0 ± 2.6	68.5 ± 3.2
20	529 ± 29	461 ± 18	58.6 ± 3.2	63.8 ± 1.3
100	367 ± 25	367 ± 63	49.6 ± 4.5	55.5 ± 12.0

a/ Mean ± standard error of weekly averages of up to 13 weeks treatment.

TABLE 4

LABORATORY DATA OF CONTROL DOGS FOR 2,6-DNT

(B,N) BASELINE
(C,N) CONTROL
N = NUMBER OF DOGS

	WKS -2-0(B, 8)	WKS 2 (C, 8)	WKS 4 (C, 8)	WKS 8 (C, 4)	WKS 13 (C, 4)
ERYTHROCYTES ($\times 10^6$ /MM^3)	6.10 ± .15	5.62 ± .18	5.69 ± .21	6.03 ± .28	5.76 ± .33
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.74 ± .08	.76 ± .07	.59 ± .08	1.01 ± .24	.56 ± .11
HEMATOCRIT, VOL, %	45.4 ± 1.1	42.1 ± 1.7	41.8 ± 1.7	46.0 ± 2.4	45.0 ± 3.5
HEMOGLOBIN, GM, %	15.3 ± .4	14.2 ± .5	14.4 ± .5	15.8 ± .8	15.7 ± 1.1
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	12.7 ± 4.1[a/]	1.3 ± 1.3
MCV, CUBIC MICRONS	74.4 ± .8	74.9 ± .8	73.4 ± .9	76.2 ± .8	77.8 ± 2.8
MCHH, MICRO MICROGMS,	25.0 ± .2	26.3 ± .2[a/]	25.3 ± .3	26.2 ± .1	27.1 ± .4[a/]
MCHBC, GM %	33.7 ± .1	35.2 ± .4[a/]	34.4 ± .2	34.4 ± .2	34.9 ± .4[a/]
PLATELETS ($\times 10^5$ /MM^3)	2.6 ± .3	2.9 ± .3	3.0 ± .3	2.9 ± .4	2.8 ± .4
LEUKOCYTES ($\times 10^3$ /MM^3)	13.0 ± .8	11.2 ± .7	11.9 ± .5	10.8 ± .6	11.2 ± .3
NEUTROPHILS, %	55.9 ± 2.7	59.3 ± 3.0	57.9 ± 2.3	55.5 ± 1.4	59.8 ± 3.6
LYMPHOCYTES, %	37.7 ± 1.9	33.6 ± 2.3	34.4 ± 2.2	35.0 ± 3.3	34.0 ± 4.0
BANDS, %	.1 ± .1	.3 ± .3	.4 ± .4	0.0 ± 0.0	.3 ± .3
EOSINOPHILS, %	4.8 ± 1.0	5.6 ± .9	5.9 ± 1.7	8.8 ± 2.7	5.3 ± 1.3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.5 ± .2	1.3 ± .4	1.5 ± .6	.8 ± .5	.8 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.1 ± .1	0.0 ± 0.0	.1 ± .1	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN,	7.8 ± .3	8.7 ± .5	10.7 ± .4[a/]	8.9 ± .5	8.1 ± .6
GLUCOSE (FASTING), MG %	98.5 ± 2.2	98.5 ± 1.9	97.5 ± 2.2	92.3 ± 3.8	82.3 ± 2.8[a/]
SGOT, IU/L	30.1 ± 1.3	24.8 ± 1.7	25.1 ± 1.4	26.3 ± 1.7	29.3 ± 2.1
SGPT, IU/L	35.7 ± 1.8	48.8 ± 10.2	35.0 ± 3.5	43.0 ± 5.5	37.8 ± 3.1
BSP, %			4.5		
ALK, PHOS,, IU/L	59 ± 5	43 ± 5[a/]	45 ± 5	41 ± 3	36 ± 3[a/]
BUN, MG %	15.1 ± .9	12.3 ± 1.2	11.1 ± 1.2	10.3 ± .6	18.3 ± 2.3
MG (MEQ/L)	1.6 ± .0	1.5 ± .0	1.4		2.1
CA (MEQ/L)	5.1 ± .0	5.3 ± .1	5.2		5.3
NA (MEQ/L)	154 ± 1	154 ± 1	146		149
K (MEQ/L)	4.6 ± .0	4.9 ± .1	4.7		4.9
CL (MEQ/L)	108 ± 0	113 ± 1	101		116

ENTRIES ARE MEAN ± STANDARD ERROR OR MEAN
WKS -2-0(B, 8) IS THE AVERAGE OF WEEKS 2, 1, AND 0 PRIOR TO TREATMENT.
a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4/]).

TABLE 5

LABORATORY DATA OF DOGS BEFORE AND DURING ADMINISTRATION OF 2,6-DNT

DOSE 4 MG/KG/DAY

(B,N) BASELINE
(T,N) TREATMENT
N = NUMBER OF DOGS

	WKS -2-0(B, A)	WKS 2 (T, A)	WKS 4 (T, A)	WKS 8 (T, 4)	WKS 13 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.57 ± .12[b]	5.06 ± .10[a]	5.26 ± .13	5.51 ± .20	5.41 ± .08
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.93 ± .08	1.10 ± .13	.98 ± .10	.88 ± .08	.62 ± .08
HEMATOCRIT, VOL, %	42.4 ± .7	38.9 ± .4	40.3 ± .9	41.8 ± 1.0	41.5 ± 1.3
HEMOGLOBIN, GM, %	14.2 ± .2	13.3 ± .2	13.6 ± .3	14.2 ± .3	14.2 ± .6
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0[b]	0.0 ± 0.0
MCV, CUBIC MICRONS	76.2 ± 1.0	76.9 ± .6	76.5 ± 1.1	75.9 ± .9	76.6 ± 1.5
MCHB, MICRO MICROGMS.	25.6 ± .3	26.3 ± .3	26.0 ± .3	25.8 ± .5	26.2 ± .9
MCHBC, GM %	33.6 ± .1	34.2 ± .3	33.9 ± .2	34.0 ± .3	34.1 ± .5
PLATELETS ($X10^5$ /MM^3)	2.7 ± .2	2.8 ± .3	2.9 ± .2	3.3 ± .4	2.8 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	13.0 ± .9	12.9 ± .8	11.7 ± .9	13.8 ± 1.0	13.5 ± 1.5
NEUTROPHILS, %	63.9 ± 2.1	67.9 ± 2.5	67.5 ± 1.2	64.3 ± 3.1	70.0 ± 3.7
LYMPHOCYTES, %	30.9 ± 2.4	27.4 ± 2.3	26.3 ± 1.4	30.8 ± 2.4	24.5 ± 4.2
BANDS, %	.2 ± .1	.4 ± .4	.5 ± .3	0.0 ± 0.0	1.0 ± .6
EOSINOPHILS, %	3.6 ± .8	3.0 ± .6[b]	4.3 ± .7	2.8 ± 1.2	3.0 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.2 ± .3	1.4 ± .2	1.4 ± .5	2.3 ± .6	1.5 ± .9
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	.1 ± .1	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.4 ± .2	.6 ± .5	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	7.6 ± .2	8.3 ± .2	8.9 ± .7	8.4 ± .3	7.8 ± .5
GLUCOSE (FASTING), MG %	97.5 ± 1.7	96.8 ± 2.7	96.6 ± 2.5	88.0 ± 4.4	82.0 ± 3.9[a]
SGOT, IU/L	27.8 ± 1.2	25.1 ± 1.4	23.5 ± 1.6	27.0 ± 3.1	25.3 ± 2.8
SGPT, IU/L	35.3 ± 2.1	33.3 ± 1.6	30.8 ± 1.8	34.8 ± 1.7	32.5 ± 1.5
BSP, %			5.5		3.0
ALK, PHOS., IU/L	48 ± 2	33 ± 3[a]	33 ± 2[a]	37 ± 4[a]	31 ± 3[a]
BUN, MG %	15.0 ± .6	12.6 ± .5[a]	11.8 ± .4[a]	10.0 ± .4[a]	12.8 ± .9
MG (MEQ/L)	1.6 ± .0	1.5 ± .0	1.5		2.1
CA (MEQ/L)	5.1 ± .0	5.3 ± .1	5.3		5.3
NA (MEQ/L)	154 ± 0	151 ± 1	144		148
K (MEQ/L)	4.7 ± .1	5.0 ± .2	4.8		4.9
CL (MEQ/L)	106 ± 1	111 ± 1	105		116

ENTRIES ARE MEAN ± STANDARD ERROR OF MEAN.
WKS -2-0(B, A) IS THE AVERAGE OF WEEKS 2, 1, AND 0 PRIOR TO TREATMENT.
a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4]).
b/ Significantly different from the control dogs at the respective time interval (Dunnett's multiple comparison procedure[4]).

TABLE 6

LABORATORY DATA OF DOGS BEFORE AND DURING ADMINISTRATION OF 2,6-DNT

DOSE 20 MG/KG/DAY

(B,N) BASELINE
(T,N) TREATMENT
N = NUMBER OF DOGS

	WKS -2-0(B, 8)	WKS 2 (T, 8)	WKS 4 (T, 8)	WKS 8 (T, 4)	WKS 9 (T, 1)[c]	WKS 13 (T, 2)
ERYTHROCYTES ($\times 10^6$ /MM^3)	6.02 ± .10	4.71 ± .20[b]	4.99 ± .19[a,b]	5.85 ± .21	3.94	4.60
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	30.11	2.05
RETICULOCYTES, %	.89 ± .08	1.43 ± .32	1.67 ± .43	.84 ± .30	.39	1.91
HEMATOCRIT, VOL, %	45.1 ± .9	39.3 ± 1.2[a]	39.0 ± 1.4[a]	45.3 ± .9	37.0	35.5
HEMOGLOBIN, GM, %	15.2 ± .3	13.0 ± .4[a]	13.0 ± .4[a]	15.0 ± .4	12.5	11.9
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 1.0[a,b]	35.2	0.0
MCV, CUBIC MICRONS	74.9 ± .4	83.6 ± 1.0[a]	78.2 ± .9	77.4 ± 1.5	93.9	77.1
MCHB, MICRO MICROGMS,	25.2 ± .2	27.7 ± .3[a,b]	26.1 ± .3	25.7 ± .5	31.7	25.5
MCHBC, GM %	33.7 ± .1	33.2 ± .1	33.4 ± .1	33.1 ± .3[b]	33.8	33.1
PLATELETS ($\times 10^5$ /MM^3)	2.3 ± .2	3.0 ± .2[a]	3.3 ± .2[a]	2.5 ± .2	1.8	2.5
LEUKOCYTES ($\times 10^3$ /MM^3)	12.4 ± .5	10.5 ± .7	9.3 ± .4[a]	10.1 ± 1.0	19.4	13.4
NEUTROPHILS, %	61.0 ± 2.1	64.4 ± 2.6	65.5 ± 2.0	60.8 ± 5.4	85.0	71.5
LYMPHOCYTES, %	34.8 ± 1.8	28.8 ± 2.9	27.6 ± 2.4	34.5 ± 5.7	12.0	24.5
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	.3 ± .2	0.0 ± 0.0	0.0	.5
EOSINOPHILS, %	2.9 ± .4	4.8 ± .7	5.1 ± 1.5	3.0 ± 1.2	0.0	1.5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0
MONOCYTES, %	1.3 ± .3	2.1 ± .5	1.5 ± .4	1.8 ± .5	3.0	2.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0
NUCLEATED RBC, %	.1 ± .1	.1 ± .1	.4 ± .4	0.0 ± 0.0	0.0	0.0
CLOTTING TIME, MIN,	8.2 ± .3	7.8 ± .8	8.6 .3[b]	9.5 ± .5	8.0	7.0
GLUCOSE (FASTING), MG %	97.6 ± 1.5	91.8 ± .6	94.4 ± 1.4	91.5 ± 4.3	111.0	66.0
SGOT, IU/L	29.2 ± 1.3	24.3 ± 1.4	24.8 ± 1.5	32.5 ± .9	62.0	35.5
SGPT, IU/L	36.3 ± 2.1	34.4 ± 2.2	31.5 ± 1.8	58.5 ± 9.8[a]	86.0	130
BSP, %			5.5			
ALK, PHOS,, IU/L	64 ± 5	48 ± 6	56 ± 8	62 ± 17	415	89
BUN, MG %	15.2 ± .5	11.5 ± .9[a]	11.5 ± 1.2[a]	10.3 ± .3[a]	11.0	15.0
MG (MEQ/L)	1.6 ± .0	1.6 ± .0	1.5		1.9	1.5
CA (MEQ/L)	5.1 ± .0	5.1 ± .1	5.1		4.8	5.2
NA (MEQ/L)	154 ± 0	151 ± 2	148		150	147
K (MEQ/L)	4.6 ± .1	5.1 ± .2	5.2		3.2	4.9
CL (MEQ/L)	108 ± 0	113 ± 1	104		109	118

ENTRIES ARE MEAN ± STANDARD ERROR OR MEAN.
WKS -2-0(B, 8) IS THE AVERAGE OF WEEKS 2, 1, AND 0 PRIOR TO TREATMENT.

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4]).

b/ Significantly different from the control dogs at the respective time interval (Dunnett's multiple comparison procedure[4]).

c/ Terminal blood from female dog No. 20 when moribund at 9 weeks.

TABLE 7

LABORATORY DATA OF DOGS BEFORE AND DURING ADMINISTRATION OF 2,6-DNT

DOSE 100 MG/KG/DAY

(B,N) BASELINE
(T,N) TREATMENT
N = NUMBER OF DOGS

	WKS -2-0(M, B)	WKS 2 (T, 7)	WKS 4 (T, 7)	WKS 5 (T, 1)[c/]	WKS 7 (T, 1)[d/]	WKS 9 (T, 1)[e/]
ERYTHROCYTES ($X10^6$ /MM^3)	5.82 ± .14	1.85 ± .28[a,b/]	2.44 ± .25[a,b/]	1.97	1.65	3.40
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.44	1.00	2.07
RETICULOCYTES, %	.92 ± .08	16.99 ± 3.33[a,b/]	6.23 ± 1.60[b/]	5.15	32.00	2.31
HEMATOCRIT, VOL, %	44.8 ± 1.1	22.9 ± 2.8[a,b/]	27.9 ± 2.4[a,b/]	19.0	20.0	31.0
HEMOGLOBIN, GM, %	15.2 ± .4	6.3 ± .9[a,b/]	8.3 ± .7[a,b/]	5.3	5.9	10.1
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	22.0	0.0
MCV, CUBIC MICRONS	77.0 ± .7[b/]	126.7 ± 5.8[a,b/]	115.9 ± 3.4[a,b/]	96.4	121.2	86.1
MCHB, MICRO MICROGMS.	26.1 ± .3[b/]	34.4 ± 1.2[a,b/]	34.5 ± 1.0[a,b/]	26.9	35.8	28.1
MCHBC, GM %	33.9 ± .2	27.3 ± .4[a,b/]	29.8 ± .6[a,b/]	27.9	29.5	32.6
PLATELETS ($X10^5$ /MM^3)	2.2 ± .2	2.8 ± .4	3.1 ± .3	3.9	4.6	2.1
LEUKOCYTES ($X10^3$ /MM^3)	13.4 ± .6	17.9 ± 3.7[b/]	20.1 ± 2.4[b/]	19.6	60.6	13.4
NEUTROPHILS, %	54.4 ± 2.3	63.9 ± 5.8	69.3 ± 5.5[b/]	51.0	77.0	71.0
LYMPHOCYTES, %	40.4 ± 1.8	30.1 ± 6.7	20.1 ± 3.8[a,b/]	40.0	11.0	23.0
BANDS, %	.1 ± .1	4.4 ± 1.3[b/]	5.1 ± 1.8[a,b/]	6.0	10.0	1.0
EOSINOPHILS, %	4.0 ± .8	.9 ± .3[a,b/]	1.3 ± .5[a,b/]	0.0	0.0	2.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0	0.0
MONOCYTES, %	1.1 ± .2	.7 ± .4	4.1 ± 1.1[a,b/]	3.0	2.0	3.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0	0.0
NUCLEATED RBC, %	.1 ± .1	19.3 ± 4.2[a,b/]	10.3 ± 3.7[b/]	8.0	4.0	4.0
CLOTTING TIME, MIN.	8.2 ± .3	7.1 ± .2	8.4 ± .6[b/]	8.0	6.0	7.0
GLUCOSE (FASTING), MG %	95.8 ± 1.9	85.6 ± 4.4[b/]	84.7 ± 4.3[b/]	177.0	128.0	123.0
SGOT, IU/L	30.6 ± 1.2	32.0 ± 3.7	28.9 ± 3.1	40.0	40.0	34.0
SGPT, IU/L	37.5 ± 3.2	74.6 ± 49.6	25.6 ± 4.2	37.0	49.0	28.0
BSP, %			7.0			
ALK, PHOS., IU/L	58 ± 4	94 ± 15[a,b/]	71 ± 7[b/]	58	189	46
BUN, MG %	16.0 ± .9	17.4 ± 2.5[b/]	14.4 ± 2.3	43.0	41.0	19.0
MG (MEQ/L)	1.6 ± .0	1.8 ± .1[a,b/]	1.7	1.6	1.7	1.9
CA (MEQ/L)	5.1 ± .1	4.8 ± .1[b/]	5.1	4.2	4.7	4.7
NA (MEQ/L)	153 ± 0	147 ± 2[a,b/]	148	152	149	140
K (MEQ/L)	4.6 ± .1	5.1 ± .3	5.4	6.1	4.7	5.8
CL (MEQ/L)	108 ± 1	114 ± 2[a/]	100	115	113	100

ENTRIES ARE MEAN ± STANDARD ERROR OF MEAN.
WKS -2-0(B, B) IS THE AVERAGE OF WEEKS 2, 1, AND 0 PRIOR TO TREATMENT.

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4/]).

b/ Significantly different from the control dogs at the respective time interval (Dunnett's multiple comparison procedure[4/]).

c/ Blood from female dog No. 32, she was moribund at the 6th week.

d/ Blood from female dog No. 31, she was moribund at the 8th week.

e/ Terminal blood from female dog No. 28 when moribund at the 8th week.

TABLE 8

LABORATORY DATA OF DOGS TREATED WITH 2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C, 2)	4 (T, 2)	20 (T, 2)	100 (T, 2)
ERYTHROCYTES ($X10^6$ /MM^3)	5.50	5.25	5.64	4.48
HEINZ BODIES, %	0.00	0.00	0.00	0.00
RETICULOCYTES, %	.86	.79	.98	1.03
HEMATOCRIT, VOL. %	41.5	42.5	42.0	36.0
HEMOGLOBIN, GM. %	14.1	14.6	14.4	11.9
METHEMOGLOBIN, %	0.0	2.9	0.0	6.0
MCV, CUBIC MICRONS	75.5	80.8	74.5	80.2
MCHB, MICRO MICROGMS.	25.7	27.7	25.6	26.5
MCHBC, GM %	34.0	34.2	34.4	33.0
PLATELETS ($X10^5$ /MM^3)	3.0	3.5	3.2	1.9
LEUKOCYTES ($X10^3$ /MM^3)	11.7	12.5	13.9	13.9
NEUTROPHILS, %	56.0	69.5	64.5	52.5
LYMPHOCYTES, %	39.0	26.5	29.5	39.0
BANDS, %	0.0	0.0	.5	1.0
EOSINOPHILS, %	4.5	4.0	5.0	7.5
BASOPHILS, %	0.0	0.0	0.0	0.0
MONOCYTES, %	.5	0.0	.5	0.0
ATYPICAL, %	0.0	0.0	0.0	0.0
NUCLEATED RBC, %	.5	0.0	0.0	1.5
CLOTTING TIME, MIN.	8.5	8.5	8.0	9.0
GLUCOSE (FASTING), MG %	90.5	93.0	98.0	97.0
SGOT, IU/L	28.0	27.5	39.5	32.5
SGPT, IU/L	29.5	35.5	44.5	83.0
ALK. PHOS., IU/L	59	42	66	35
BUN, MG %	9.0	8.5	9.0	17.0
MG (MEQ/L)	1.5	1.6	1.5	
CA (MEQ/L)	5.1	5.1	5.3	
NA (MEQ/L)	145	147	147	
K (MEQ/L)	4.8	5.2	4.5	
CL (MEQ/L)	112	107	109	

ENTRIES ARE MEAN

TABLE 9

LABORATORY DATA OF DOGS TREATED WITH 2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 19 WEEKS

DOSE 100 MG/KG/DAY (R,N) RECOVERY N = NUMBER OF DOGS

	WKS 8 (R, 2)	WKS 13 (R, 2)	WKS 23 (R, 2)
ERYTHROCYTES ($X10^6$ /MM^3)	4.68	5.02	6.00
HEINZ BODIES, %	0.00	0.00	0.00
RETICULOCYTES, %	1.03	1.05	.44
HEMATOCRIT, VOL. %	36.0	37.5	42.0
HEMOGLOBIN, GM, %	11.9	12.6	14.6
METHEMOGLOBIN, %	6.0	0.0	0.0
MCV, CUBIC MICRONS	80.2	74.7	70.0
MCHB, MICRO MICROGMS.	26.5	25.2	24.2
MCHBC, GM %	33.0	33.7	34.6
PLATELETS ($X10^5$ /MM^3)	1.9	2.3	2.2
LEUKOCYTES ($X10^3$ /MM^3)	13.9	14.4	12.4
NEUTROPHILS, %	52.5	68.0	67.0
LYMPHOCYTES, %	34.0	26.0	27.0
BANDS, %	1.0	0.0	0.0
EOSINOPHILS, %	7.5	2.0	4.0
BASOPHILS, %	0.0	0.0	0.0
MONOCYTES, %	0.0	4.0	2.0
ATYPICAL, %	0.0	0.0	0.0
NUCLEATED RBC, %	1.5	0.0	0.0
CLOTTING TIME, MIN.	9.0	7.5	5.3
GLUCOSE (FASTING), MG %	97.0	87.5	100.0
SGOT, IU/L	32.5	37.0	19.5
SGPT, IU/L	83.0	54.0	41.5
ALK. PHOS., IU/L	35	37	26
BUN, MG%	17.0	14.0	10.5
MG (MEQ/L) IU/L		1.5	1.5
CA (MEQ/L)		5.2	4.7
NA (MEQ/L)		151	147
K (MEQ/L)		4.8	4.9
CL (MEQ/L)		121	

ENTRIES ARE MEAN

TABLE 10

LABORATORY DATA OF DOGS TREATED WITH 2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

	(C,N) CONTROL	(T,N) TREATED	N = NUMBER OF DOGS
DOSE: MG/KG/DAY	0 (C, 2)	4 (T, 2)	20 (T, 1)
ERYTHROCYTES ($X10^6$ /MM^3)	6.40	5.86	5.84
HEINZ BODIES, %	0.00	0.00	0.00
RETICULOCYTES, %	.83	.84	.63
HEMATOCRIT, VOL, %	46.0	40.5	41.0
HEMOGLOBIN, GM, %	15.8	14.4	14.1
METHEMOGLOBIN, %	2.8	0.0	0.0
MCV, CUBIC MICRONS	70.3	69.1	70.2
MCHH, MICRO MICROGMS,	24.8	24.6	24.1
MCHBC, GM %	35.2	35.6	34.4
PLATELETS ($X10^5$ /MM^3)	2.8	2.5	2.5
LEUKOCYTES ($X10^3$ /MM^3)	12.2	16.0	10.4
NEUTROPHILS, %	57.5	69.0	68.0
LYMPHOCYTES, %	34.5	28.5	25.0
BANDS, %	0.0	0.0	0.0
EOSINOPHILS, %	5.5	1.0	6.0
BASOPHILS, %	0.0	0.0	0.0
MONOCYTES, %	2.5	1.5	1.0
ATYPICAL, %	0.0	0.0	0.0
NUCLEATED RBC, %	0.0	0.0	0.0
CLOTTING TIME, MIN,	5.8	5.5	6.0
GLUCOSE (FASTING), MG %	79.0	94.5	78.0
SGOT, IU/L	24.0	26.0	18.0
SGPT, IU/L	32.5	32.5	37.0
BSP, %	5	4	8
ALK, PHOS,, IU/L	35	37	55
BUN, MG %	16.5	14.5	9.0
MG (MEQ/L)	1.4	1.4	1.3
CA (MEQ/L)	4.6	4.9	4.5
NA (MEQ/L)	141	140	137
K (MEQ/L)	4.9	5.4	5.1
CL (MEQ/L)	114	114	113

ENTRIES ARE MEAN

TABLE 11

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS TREATED WITH 2,6-DNT FOR 4 WEEKS

Dose (mg/kg/day)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weight (gm)								
			Heart	Liver	Kidneys	Spleen	Adrenals	Thyroid	Testes	Ovaries	Brain
0	7	12.8	68.3	498	60.9	30.1	1.54	0.99	20.2	--	89.0
0	8	8.0	45.0	306	42.0	10.4	1.04	0.78	--	1.04	70.5
4	15	12.5	81.5	542	70.0	40.0	1.54	1.41	19.0	--	b/
4	16	8.2	51.0	257	45.5	25.0	0.97	0.78	--	0.97	71.0
20	23	10.4	78.0	335	47.0	38.5	1.11	0.89	14.0	--	86.5
20	24	8.4	63.0	328	47.0	28.0	0.97	0.93	--	0.97	77.0
100	25	7.2a/	78.0	365	42.0	65.0	1.13	0.66	13.2	--	78.0
100	26	5.6	48.0	241	39.5	98.0	1.18	0.58	--	0.65	68.5

		Relative Organ Weight (gm/kg Body Weight)								
		Heart	Liver	Kidneys	Spleen	Adrenals	Thyroid	Testes	Ovaries	Brain
0	7	5.34	38.9	4.76	2.35	0.120	0.077	1.58	--	6.95
0	8	5.63	38.3	5.25	1.30	0.103	0.098	--	0.130	8.81
4	15	6.52	36.2	5.60	3.20	0.126	0.113	1.52	--	b/
4	16	7.07	31.3	5.55	3.05	0.118	0.095	--	0.118	8.66
20	23	7.50	32.2	4.52	3.70	0.107	0.086	1.35	--	8.32
20	24	7.50	39.0	5.60	3.33	0.116	0.111	--	0.126	9.17
100	25	10.83	50.7	5.83	9.03	0.150	0.092	1.83	--	10.83
100	26	8.57	43.0	7.05	17.50	0.211	0.104	--	0.116	12.23

		Relative Organ Weight (gm/gm Brain Weight)							
		Heart	Liver	Kidneys	Spleen	Adrenals	Thyroid	Testes	Ovaries
0	7	0.767	5.59	0.684	0.338	0.0173	0.0111	0.227	--
0	8	0.638	4.35	0.596	0.148	0.0116	0.0111	--	0.0148
4	16	0.816	3.61	0.641	0.352	0.0137	0.0110	--	0.0137
20	23	0.902	3.87	0.543	0.445	0.0128	0.0103	0.162	--
20	24	0.818	4.25	0.610	0.364	0.0126	0.0121	--	0.0138
100	25	1.000	4.68	0.539	0.833	0.0145	0.0085	0.169	--
100	26	0.701	3.52	0.577	1.431	0.0172	0.0085	--	0.0095

a/ Moribund and terminated during week 2

TABLE 12

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS TREATED WITH 2,6-DNT FOR 13 WEEKS

Dose (mg/kg/day)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weights (gm)								
			Heart	Liver	Kidneys	Spleen	Adrenals	Thyroid	Testes	Ovaries	Brain
0	3	10.8	69.0	407	54.0	52.0	0.94	0.85	15.0	--	85.0
0	4	7.7	65.0	246	39.0	30.4	0.90	0.74	--	2.18	75.0
4	11	12.0	71.0	353	54.0	26.0	e/	e/	13.0	--	86.0
4	12	9.6	70.0	377	47.0	26.0	1.24	0.87	--	0.55	85.7
20	18	6.0a/	57.6	242	55.0	19.7	e/	e/	--	0.55	85.7
20	19	11.4	66.0	320	53.0	34.0	1.23	0.84	15.0	--	87.0
20	20	5.2a/	37.8	208	45.6	24.0	1.11	0.45	--	0.65	72.2
100	32	4.9b/	48.0	206	44.0	48.0	1.37	0.75	--	1.05	64.0
100	27	6.0c/	84.5	308	64.0	24.2	1.37	0.66	4.71	--	49.6
100	28	6.6d/	42.9	221	39.0	41.0	1.16	0.53	--	0.75	71.0
100	31	6.7d/	58.0	337	51.0	57.5	1.38	0.67	4.80	--	84.0

Dose (mg/kg/day)	Dog No.	Relative Organ Weight (gm/kg Body Weight)								
		Heart	Liver	Kidneys	Spleen	Adrenals	Thyroid	Testes	Ovaries	Brain
0	3	6.39	37.7	5.00	4.81	0.087	0.079	1.39	--	7.87
0	4	8.31	31.9	5.06	3.95	0.096	0.096	--	0.283	9.74
4	11	7.40	29.4	4.50	2.17	e/	e/	1.08	--	7.17
4	12	7.29	39.3	4.90	2.71	0.129	0.091	--	0.255	7.81
20	18	9.60	40.3	9.17	3.28	e/	e/	--	0.092	14.28
20	19	5.79	28.1	4.65	2.98	0.108	0.074	1.32	--	7.63
20	20	7.27	40.0	8.77	4.62	0.214	0.087	--	0.125	13.88
100	32	9.80	42.0	8.98	9.80	0.280	0.153	--	0.214	13.06
100	27	14.08	51.3	10.67	4.03	0.228	0.110	0.79	--	8.27
100	28	6.50	33.4	5.91	6.21	0.176	0.080	--	0.114	10.76
100	31	8.66	50.2	7.61	8.58	0.206	0.100	0.72	--	12.54

Dose (mg/kg/day)	Dog No.	Relative Organ Weight (gm/gm Brain Weight)							
		Heart	Liver	Kidneys	Spleen	Adrenals	Thyroid	Testes	Ovaries
0	3	0.812	4.79	0.635	0.612	0.0111	0.0100	0.177	--
0	4	0.853	3.28	0.520	0.405	0.0120	0.0099	--	0.0291
4	11	0.826	4.10	0.628	0.302	e/	e/	0.151	--
4	12	0.933	5.03	0.627	0.347	0.0165	0.0116	--	0.0327
20	18	0.672	2.82	0.642	0.230	e/	e/	--	0.0062
20	19	0.759	3.68	0.609	0.391	0.0141	0.0097	0.172	--
20	20	0.524	2.88	0.632	0.332	0.0154	0.0062	--	0.0090
100	32	0.750	3.22	0.688	0.750	0.0214	0.0117	--	0.0164
100	27	1.704	6.21	1.290	0.488	0.0276	0.0133	0.095	--
100	28	0.604	3.12	0.549	0.578	0.0163	0.0075	--	0.0106
100	31	0.691	4.01	0.607	0.685	0.0164	0.0080	0.057	--

a/ Moribund and terminated during week 9
b/ Moribund and terminated during week 6
c/ Moribund and terminated during week 7
d/ Moribund and terminated during week 8
e/ Not recorded

TABLE

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS TREATED WITH 2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Dose (mg/kg)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weight (gm)								
			Heart	Liver	Kidneys	Spleen	Adrenals	Thyroid	Testes	Ovaries	Brain
0	5	8.7	55.7	290	47.6	12.5	0.99	6.77	14.0	--	83.5
0	6	9.4	65.0	483	49.7	26.8	1.01	0.78	--	1.76	70.4
4	13	11.5	57.0	465	62.4	21.3	1.08	1.39	16.6	--	91.0
4	14	8.4	60.3	312	46.0	32.0	0.92	0.81	--	2.28	72.5
20	21	10.0	59.6	351	48.2	18.0	1.29	1.28	11.2	--	73.4
20	22	9.3	68.4	348	54.7	28.0	1.15	1.15	--	0.77	81.0

Dose (mg/kg)	Dog No.	Relative Organ Weight (gm/kg Body Weight)								
		Heart	Liver	Kidneys	Spleen	Adrenals	Thyroid	Testes	Ovaries	Brain
0	5	6.40	33.3	5.47	1.44	0.114	0.089	1.61	--	9.60
0	6	6.91	51.4	5.29	2.85	0.107	0.083	--	0.187	7.49
4	13	4.96	40.5	5.43	1.85	0.094	0.121	1.44	--	7.91
4	14	7.18	37.1	5.48	3.81	0.110	0.096	--	0.271	8.63
20	21	5.96	35.2	4.82	1.80	0.129	0.128	1.12	--	7.34
20	22	7.35	37.4	5.88	3.01	0.124	0.124	--	0.083	8.71

Dose (mg/kg)	Dog No.	Relative Organ Weight (gm/gm Brain Weight)							
		Heart	Liver	Kidneys	Spleen	Adrenals	Thyroid	Testes	Ovaries
0	5	0.667	3.47	0.570	0.150	0.0119	0.0092	0.167	--
0	6	0.923	6.86	0.706	0.381	0.0143	0.0111	--	0.0250
4	13	0.626	5.12	0.686	0.234	0.0119	0.0153	0.182	--
4	14	0.832	4.30	0.635	0.441	0.0127	0.0112	--	0.0314
20	21	0.812	4.79	0.657	0.245	0.0176	0.0174	0.153	--
20	22	0.844	4.30	0.675	0.346	0.0142	0.0142	--	0.0095

TABLE 14

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS TREATED WITH 2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Dose (mg/kg)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weight (gm)								
			Heart	Liver	Kidneys	Spleen	Adrenals	Thyroid	Testes	Ovaries	Brain
0	1	10.7	59.0	294	51.5	33.0	1.44	0.85	16.0	--	92.5
0	2	9.1	66.5	263	40.0	24.5	1.38	0.70	--	2.00	71.0
4	9	12.7	78.5	421	62.0	40.5	1.33	1.24	21.0	--	86.0
4	10	11.2	68.5	432	53.5	36.5	1.27	0.97	--	1.60	80.5
20	17	8.6	69.5	290	46.0	32.0	1.44	0.69	8.0	--	73.0
			Relative Organ Weight (gm/kg Body Weight)								
			Heart	Liver	Kidneys	Spleen	Adrenals	Thyroid	Testes	Ovaries	Brain
0	1		5.51	27.5	4.81	3.08	0.135	0.079	1.50	--	8.64
0	2		7.31	28.9	4.40	2.69	0.152	0.077	--	0.220	7.80
4	9		6.18	33.1	4.88	3.19	0.105	0.098	1.65	--	6.77
4	10		6.12	38.6	4.78	3.26	0.113	0.087	--	0.143	7.19
20	17		8.08	33.7	5.35	3.72	0.167	0.080	0.93	--	8.49
			Relative Organ Weight (gm/gm Brain Weight)								
			Heart	Liver	Kidneys	Spleen	Adrenals	Thyroid	Testes	Ovaries	
0	1		0.638	3.17	0.557	0.357	0.0156	0.0092	0.173	--	
0	2		0.937	3.70	0.563	0.345	0.0194	0.0099	--	0.0282	
4	9		0.913	4.90	0.721	0.471	0.0155	0.0144	0.244	--	
4	10		0.851	5.37	0.665	0.453	0.0158	0.0120	--	0.0199	
20	17		0.952	3.97	0.630	0.438	0.0197	0.0095	0.110	--	

TABLE 15

SUMMARY OF TISSUE LESIONS IN DOGS TREATED WITH 2,6-DNT FOR 4 WEEKS

Lesions[a] / Dog No.:	Dose (mg/kg/day) 0		4		20		100	
	7	8	15	16	23	24	25[b]	26
Lung								
Focal inflammation	2	2			3			1
Liver								
Extramedullary hematopoiesis					1		1	3
Bile duct hyperplasia						1		
Degeneration							2	
Subacute inflammation						1	2	
Testis								
Degeneration and/or retardation of spermatogenesis					1		2	
Ovary								
Several graafian follicles, but no corpora lutea				+		+		+
Thyroid								
Increase in parafollicular cells			1		1	1		1
Spleen								
Extramedullary hematopoiesis				1	2	2	1	4
Lymphoid depletion							2	
Tonsil								
Inflammation			1					2
Lymph Node								
Lymphoid degeneration and depletion							2	
Thymus								
Involution								2
Focal hyaliniztion of the corpuscles								1
Bone Marrow								
M/E Ratio	1.4	1.3	1.3	1.1	1.1	1.1	--	0.9

Tissues not listed were normal.

a/ Severity of lesions: + = present; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

b/ Died after 12 days treatment.

TABLE 16

SUMMARY OF TISSUE LESIONS IN DOGS TREATED WITH 2,6-DNT FOR 13 WEEKS

Lesions[a]	Dose (mg/kg/day) 0		4		20			100			
Dog No.:	3	4	11	12	18[b]	19	20[b]	27[c]	28[d]	31[d]	32[e]
Lung											
Focal bronchopneumonia											2
Focal inflammation	2	1	1	2		±		2		2	
Heartworm							1				
Tongue											
Focal glossitis				1							
Liver											
Extramedullary hematopoiesis					2	1	1	3	3	2	3
Bile duct hyperplasia						1	2	2			±
Degeneration					2	2	1	1	2	2	2
Subacute inflammation					2	1					
Gall Bladder											
Epithelial hyperplasia								1			
Pancreas											
Inflammation						±	2				
Kidney											
Dilated tubules								2			1
Foci of inflammation											1
Focal degeneration					1	1					
Yellow pigment and/or casts in tubules						1		2		2	
Testis											
Atrophy and aspermatogenesis								3		2	
Ovary											
Several graafian follicles, but no corpora lutea					+		+		+		
Cystic corpus lutea				1							
Adrenal											
Focal vacuolation	1							1			1
Thyroid											
Increase in parafollicular cells		1	1			2	2	1		1	2
Pituitary											
Cysts				1							
Thymus											
Involution											3
Spleen											
Extramedullary hematopoiesis			1	±	2	±	1	4	3	3	4
Lymphoid depletion				2	1						
Focal fibrosis						2					
Tonsil											
Inflammation	1	1	1			1					
Lymph node											
Inflammation	2	3	1	2		2	2			2	
Lymphoid depletion and degeneration								3		2	2
Bone Marrow											
M/E Ratio	1.3	1.5	1.4	1.3	--	1.3	1.2	1.0	1.0	0.9	1.0

Tissues not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

b/ Died during week 9.

c/ Died during week 7.

d/ Died during week 8.

e/ Died during week 6.

TABLE 17

SUMMARY OF TISSUE LESIONS IN DOGS TREATED WITH 2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a] / Dog No.:	Dose (mg/kg/day) 0		4		20		100	
	5	6	13	14	21	22	29[b]	30[b]
Lung								
Focal inflammation					1		1	2
Liver								
Extramedullary hematopoiesis					±	±		
Hemosiderosis							1	1
Gall Bladder								
Increase in lymphoid tissue			1	1				
Kidney								
Focal degeneration								1
Testis								
Degeneration and retardation of spermatogenesis					1			
Ovary								
Several graafian follicles, but no corpora lutea						+		+
Thyroid								
Increasing parafollicular cells	1		1	1	1			
Spleen								
Extramedullary hematopoiesis		±		±	1			
Bone Marrow								
M/E Ratio	1.4	1.4	1.5	1.4	1.4	1.3	1.3	1.4

Tissues not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

b/ Allowed to recover for 19 weeks.

TABLE 18

SUMMARY OF TISSUE LESIONS IN DOGS TREATED WITH 2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a/]	Dose (mg/kg/day) 0		4		20
Dog No.:	1	2	9	10	17
Heart					
Focal, fatty ingrowth	1				
Lung					
Focal inflammation	1	1	1	1	
Liver					
Hemosiderosis					2
Bile duct hyperplasia				±	3
Biliary fibrosis					3
Subacute inflammation	±	±		1	2
Gall Bladder					
Inflammation and thickening					1
Kidney					
Focal degeneration					1
Yellow pigment in tubules					1
Testis					
Atrophy and aspermatogenesis					2
Ovary					
Cystic corpus lutea		1			
Pituitary					
Cysts					1
Thyroid					
Increase in parafollicular cells	1	1			
Spleen					
Focal fibrosis		1			
Hemosiderosis		1		±	1
Thymus					
Focal hyalinization of the corpuscles	1				
Bone Marrow					
M/E Ratio	1.4	1.3	1.3	1.5	1.3

Tissues not listed were normal.

a/ Severity of lesions: ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

TABLE 19

SERUM IgE (IU/ml) OF DOGS TREATED WITH 2,6-DNT

Dose (mg/kg/day)	Treatment Weeks			
	Week 0	Week 2	Week 4	Week 13
0	1437 ± 35(8)[a]	1560 ± 99(5)	1328 ± 71(8)	988(2)
4	1379 ± 45(8)	[b]	1197 ± 51(8)	1275(2)
20	1399 ± 30(7)	[b]	1409 ± 47(8)	[b]
100	1375 ± 11(8)	1479 ± 49(7)	1386 ± 40(7)	[b]

[a] Mean ± Standard Error or Mean (number of dogs).
[b] No samples tested.

II. RATS

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II. RATS

A. Subacute and Subchronic Toxicities and Reversibility

1. Introduction

As for the dogs, these studies were performed to define the nature and extent of effects of 2,6-DNT on the biological system at the biochemical and cellular levels and to elucidate the dose-response relationship in rats fed 2,6-DNT for 4 weeks and 13 weeks. The reversibility of any adverse effects was also studied after the feeding of 2,6-DNT was discontinued for 4 weeks.

2. Material and Methods

a. Number of Rats, Sex and Treatment

A total of 64 male and 64 female young healthy CD® rats (Charles River Breeding Lab.) were used for this study. They were divided into four groups, each consisting of 16 males and 16 females. The average weights of all groups were kept close. Three groups of rats were fed 0.01, 0.05 or 0.25% of 2,6-DNT in the feed. The 4th group, serving as the controls, was given the powdered standard rodent chow (Wayne Laboratory Meal) without 2,6-DNT.

b. Animal Husbandry

Our animal quarters have a ventilation system with 10 air changes per hour. The room air is passed through filters to remove 99.9% of all particles larger than 0.3 μ. The temperature is maintained at 75 ± 5°F and the relative humidity at 50 ± 10%. All animal rooms were kept at 12-hour light cycles.

Upon arrival from the breeder, the rats were isolated and conditioned in our rodent quarter for at least 2 weeks before starting on the experiment. They were housed two per plastic cage with filter tops. Hardwood chips were used after steam-sterilization as bedding material and changed weekly. All cages, cover tops and water bottles were steam-sterilized before using and once every month. Feed and water were available at all times.

c. Feed Preparation

2,6-DNT was obtained from K & K Laboratories (Cleveland, OH) and added to feed to produce a diet containing the desired 0.25% of 2,6-DNT. The diet was placed in a wooden box (16 x 16 x 20 inches) until

half full and rotated in a modified cement mixer about its long axis for 1 hour at a speed of 20 rpm. Subsequently, this diet was mixed with the standard chow at 20% by weight to yield 0.05% and again 0.01% of 2,6-DNT, respectively.

d. Experimental Procedure

The experimental procedure for rats was the same as for dogs, described in Section I.A.2.b., with the following exceptions:

(1) Feed consumption of all rats was measured throughout the experiment.

(2) Blood samples were collected by cutting the tip of the tail at 0, 4, 8, 13 and/or 17 weeks for hematology tests. In addition, terminal blood was collected from the abdominal aorta under ether anesthesia for clinical chemistry tests.

(3) BSP retention test was not performed.

e. Experimental Desi[illegible]

The experimental design for rats was the same as for dogs, described in Section I.A.2.c., with the following exceptions:

(1) At the end of 4 and 13 weeks, four male and four female rats from each group were euthanized for necropsy. Liver, spleen, kidneys, heart and brain were weighed.

(2) The treatment for four male and four female rats from each group was discontinued at the end of 4 weeks. They were kept for observation and necropsied for examination after an additional 4 weeks.

(3) Similarly, the treatment for four male and four female rats from each group was discontinued at the end of 13 weeks. These rats were euthanized for necropsy at the end of 17 weeks to study the reversibility of any adverse effects.

3. Results

a. General Observations and Weight Gain

Unlike the dogs described above, there were no overt neuromuscular symptoms. The high dosage level rats were less active. They had rough coats and similar signs of malnutrition. There were no specific symptoms and no unscheduled deaths.

The body weights of the male and female rats before, during and after treatment are summarized in Tables 20 and 21, respectively. The weight changes are better illustrated in Figure 1. Rats fed the low level of 2,6-DNT (0.01% in feed) had somewhat less weight gain than the controls. Rats fed the middle (0.05%) and high (0.25%) levels had greater depression in weight gain. Some of the high dosage animals actually lost weight. During the recovery period, most rats gained weight, with the lowest weight ones gaining the most. Some individuals continued to lose weight during part of the recovery period, showing little total change from week 13 until week 17.

b. Feed Consumption and 2,6-DNT Intake

The feed consumption of the rats fed various levels of 2,6-DNT is summarized in Table 22. The data generally reflect the weight gain data, but scattering of feed by the rats occasionally caused artificially high apparent consumption. All female rats fed 2,6-DNT and males fed 0.05% or 0.25% of 2,6-DNT in feed ate less than controls, with this amount of feed consumption in reverse proportion to the amount of 2,6-DNT in the feed. During the recovery periods, these rats usually ate more, but not as much as the controls.

The 2,6-DNT intake of these rats fluctuated slightly during the experiment and is summarized in Table 23. The intake of the male and female rats fed 0.01% of 2,6-DNT averaged 7.2 or 7.4 mg/kg/day, respectively; and that of those fed 0.05% averaged 35.1 or 37.1 mg/kg/day, respectively. Due to increasing feed consumption and low weight gain, the 2,6-DNT intake of rats fed 0.25% increased throughout the study, averaging 144.7 and 155.0 mg/kg/day for the males and females, respectively.

c. Blood Analysis

The hematology results from the male control rats and males fed various levels of 2,6-DNT are summarized in Tables 24 through 27. The peripheral blood elements of the male controls fluctuated within normal limits (Table 24). However, they had statistically significant decreases in reticulocyte count and statistically significant increases in platelet count. These changes were of no clinical importance. The males fed the low (0.01%) and middle (0.05%) levels showed similar fluctuations and changes (Tables 25 and 26) as seen in the male controls. In addition, the males fed the middle level had increases in erythrocyte count and hematocrit at 4 and 13 weeks, and increases in leukocyte count with neutrophilia and corresponding lymphopenia at 13 weeks. The erythrocytosis and increase in hematocrit were mild and were not seen in male controls or males fed the low or high level of 2,6-DNT. The males fed the high level (0.25%) showed a decrease in erythrocyte count with a compensatory reticulocytosis and an increase in leukocyte count at 4 weeks (Table 27). These

parameters partially recovered at 8 or 13 weeks. Small amounts of methemoglobin were also present in the blood of the high dosage males at 8 or 13 weeks. When the males were allowed to recover after treated for 4 or 13 weeks, the changes seen in the middle and/or high dosage male disappeared (Tables 28 and 29).

Clinical laboratory data from the rats terminated after various treatment periods are shown in Tables 30 through 33, respectively. SGPT was elevated in some males fed the high level of 2,6-DNT. The fluctuations seen in alkaline phosphatase, BUN and electrolytes were small and within normal variations.

The hematology results from the female control rats and rats fed various levels of 2,6-DNT are summarized in Tables 34 to 37, respectively. As seen in the males, the female controls and females fed the low and middle level had a decrease in reticulocyte count; the females fed the middle level had leukocytosis; the females fed the high level had reticulocytosis and leukocytosis at 4 and 8 weeks, but not at 13 weeks. Also as seen in the males, methemoglobin appeared in the blood of females fed the high level at 13 weeks. When allowed to recover for 4 weeks, the various peripheral blood elements of the females fed the middle and high levels of 2,6-DNT returned within normal limits (Tables 38 and 39).

Clinical laboratory data from female rats terminated at various times are summarized in Tables 40 through 43. As seen in the males, the SGPT was elevated in the females fed the high level of 2,6-DNT for 4 weeks and in some females fed the high level for 13 weeks. The SGPT returned to normal ranges when the females were allowed to recover for 4 weeks. The other changes in alkaline phosphatase and serum magnesium and sodium were mild and inconsistent; they were not related to 2,6-DNT.

d. Organ Weights

The organ weights of rats fed various levels of 2,6-DNT are summarized in Tables 44 through 47. After 4 or 13 weeks, the absolute organ weights of the high and/or the low dosage rats were usually less than those of the controls. Based on the body or brain weight, they were equal or greater than those of the controls. The differences are presumably due to the general effect of malnutrition rather than a specific toxic effect on an organ. The rats fed 2,6-DNT for 4 or 13 weeks and allowed to recover for 4 weeks showed partial recovery in their body weight. Accordingly, less changes in absolute or relative organ weights were seen.

e. Gross and Microscopic Examination of Tissues

The control rats and those fed the low dose level (0.01%) were in good nutritional condition at the time of necropsy. Most rats fed the middle and high doses were in poor nutritional condition with little or no visible fat.

Male and female control rats had a number of spontaneous lesions (Tables 48 through 51). These lesions included chronic murine pneumonia and/or perivasculitis in the lung or focal mononuclear infiltration in the liver. Other occasional lesions included a focal hemorrhage in the brain, a focal myocarditis and tracheitis or a focal atrophy in the testes of the male controls (Tables 48 and 50), minimal microcalculi and focal mononuclear infiltration in the kidney, eosinophil infiltration in the uterus and/or hemosiderosis in the spleen of the female controls (Tables 49 and 51). These lesions were seen more often in the rats fed various levels of 2,6-DNT. In addition, the middle (0.05%) and high (0.25%) levels of 2,6-DNT caused degeneration and atrophy of the testes and depression of spermatogenesis in the males (Tables 48 and 50), bile duct hyperplasia in the liver, extramedullary hematopoiesis in the spleen and liver, and hemosiderosis in the spleen of both the males and the females (Tables 48 through 51). In general, these lesions were more frequent and more severe in rats fed the high level of 2,6-DNT than in those fed the middle level and in rats fed 2,6-DNT for 13 weeks than those fed for 4 weeks. The testes of rats fed the high level of 2,6-DNT for 13 weeks consisted of virtually all connective tissue and were practically completely devoid of spermatogenesis (Table 50). The spleen of these rats contained large amounts of hemosiderin. Low level (0.01%) of 2,6-DNT also caused extramedullary hematopoiesis and/or increased hemosiderin in the spleen of the females fed for 4 weeks (Table 49) and of both the males and the females fed for 13 weeks (Tables 50 and 51). The bone marrows of these rats were normal, and their M/E ratios of the rib marrows were within normal ranges.

Both the male and female rats fed 2,6-DNT for 4 weeks and allowed to recover for 4 weeks had partial recovery in lesions (Tables 52 and 53). Bile duct hyperplasia and extramedullary hematopoiesis were seen in the livers of some rats. Portions of the seminiferous tubules of one rat (No. 186, Table 52) were beginning to regenerate although the remaining tubules were fibrotic with an increase of interstitial tissue. Partial recovery was also seen in rats fed 2,6-DNT for 13 weeks and allowed to recover for 4 weeks (Tables 54 and 55). One control rat (No. 102, Table 54) had marked atrophy and aspermatogenesis with some normal seminiferous tubules. This lesion was incidental and was different from the complete aspermatogenesis caused by 2,6-DNT.

4. Discussion and Conclusions

The 2,6-DNT intake of the male rats fed the low, middle or high level of 2,6-DNT in the feed averaged 7.2, 35.1 and 144.7 mg/kg/day, respectively. The 2,6-DNT intakes of the corresponding female rats averaged 7.4, 37.1 and 155.0 mg/kg/day, respectively.

Male and female rats fed the low level of 2,6-DNT for up to 13 weeks were not affected. Weight gain was decreased, but this was not statistically significant. There was mild hemosiderosis in some spleens, but this is common in rats.

Male and female rats fed the middle level had decreased weight gain and a corresponding decreased feed intake. SGPT was elevated in some rats. Three characteristic lesions were observed These were extramedullary hematopoietic activity in the spleen and/or liver, bile duct hyperplasia, and/or depression of spermatogenesis and atrophy in the testes.

The rats fed the high level of 2,6-DNT were more severely affected. Some rats had weight loss instead of depressed weight gain. There was a decrease in erythrocyte count with compensatory reticulocytosis. The detection of methemoglobin and Heinz bodies indicated a toxic methemoglobin-induced anemia. By the end of 13 weeks, the testicular lesions had progressed to consist of virtually all connective tissue. The bile duct hyperplasia and extramedullary hematopoiesis were more prevalent and more severe.

Some adaptation apparently took place during continued 2,6-DNT intake. This was apparent from the increasing amounts of feed and 2,6-DNT intake and decreasing degree of anemia from week 4 to week 13 in the high dosage rats. However, tissue lesions, particularly in the testes, worsened during the course of treatment. The rats were only able to recover partially from these effects when the treatment was discontinued for 4 weeks.

As with the dogs, the effects of 2,6-DNT were quantitatively and qualitatively similar to those previously reported for 2,4-DNT.[6] The main effects, on the erythrocytes and testes, were virtually identical. In addition, 2,6-DNT produced bile duct hyperplasia.

B. Cytogenetic and Mutagenic Effects of 2,6-DNT

1. Introduction

The cytogenetic effect of 2,6-DNT on somatic cell chromosomes was studied. The lymphocyte and kidney cultures from rats fed 2,6-DNT were obtained and examined for any damages. In addition, the capability of 2,6-DNT to induce single gene mutations was studied in Chinese hamster ovary cells in vitro. The Chinese hamster ovary system is capable of detecting mutations induced in nine different specific loci simultaneously.

2. Material and Methods

a. Animals

Control rats and rats fed the middle level (0.05%) of 2,6 DNT from the toxicity study and other rats treated identically were used in the cytogenetic studies.

b. Lymphocyte and Kidney Cultures

At the end of 6 and 13 weeks, blood samples were aseptically drawn from the jugular vein of both the control and treated rats. The lymphocytes were cultured by the method of Moorhead et al.[9] Kidney tissue samples were removed at necropsy and cultured by the trypsinization method of Fernandes.[10] All cultures were maintained in Eagle's medium as modified by Vogt and Dulbecco.[11]

c. Chromosome Analysis

Actively dividing kidney cultures and phytohemagglutin-stimulated lymphocytes were arrested in metaphase by short-term colchicine treatment. The cells were trypsinized, swollen in hypotonic solution, and processed for spreading on glass slides by the method of Moorhead and Nowell.[12] Slides were stained with giemsa and scanned under low power optics. Cell polyploidy was estimated by examination of 200 cells. Slides showing minimum scattering of cells in metaphase were selected for analysis under oil immersion optics. Chromosomes were counted and morphological aberrations were examined from photographic negatives of up to 50 metaphase cells.

d. Mutagenic Effects on CHO-K1 Cells In Vitro

Wild type Chinese hamster ovary (CHO-K1) cells[13] capable of growth in both a minimal and an enriched medium were exposed to selected concentration of 2,6-DNT to test its ability to induce single gene mutations in mammalian somatic cells. The concentrations used were selected from a single cell survival curve obtained according to the method of Puck and Kao.[14] Potential mutants were isolated by the BUdR-visible light technique and confirmed by plating the cells in both media. A mutant was defined as having the capability of growth only in the enriched medium and not in the minimal medium. Mutagenesis was measured relative to the known mutagen ethyl methanesulfonate.[15]

3. Results

The results on numerical and morphological aberrations of chromosomes are shown in Tables 56 and 57, respectively. Kidney cultures from

rats fed 0.05% of 2,6-DNT for 13 weeks (35 to 37 mg/kg/day) had an increased number of tetraploid chromosomes. Lymphocyte cultures from rats fed 0.05% of 2,6-DNT for either 6 or 13 weeks had an increased number of chromatid breaks and gaps. The preliminary experiments with Chinese hamster ovary cultures found the 1% and 5% cell survival concentrations to be 112 µg/ml and 77.5 µg/ml, respectively. Neither of these concentrations caused mutations, although comparable concentrations of the known mutagen ethyl methanesulfonate did cause mutations.

4. Discussion and Conclusions

The middle level of 2,6-DNT in the feed (about 35 mg/kg/day) for 6 weeks increased the number of chromatid breaks and gaps in lymphocyte culture. Continuing the feeding for 13 weeks caused an increase in tetraploids in kidney cultures, in addition to the lymphocyte culture effect.

2,6-DNT was not mutagenic in the specific locus mutation test using Chinese hamster ovaries.

C. Immunologic Response to 2,6-DNT

1. Introduction

Immunoglobin E (IgE), the allergic or hypersensitive antibody, was associated with anaphylactic reactions in humans.[7] Serum concentration of IgE of rats treated with 2,6-DNT was determined.

2. Material and Method

As described for the dogs in Section I.B.2., the immunodiffusion technique of Mancini[8] was used to determine the serum IgE of control rats and rats fed 0.25% of 2,6-DNT. Samples of blood taken at terminations of the rats in the toxicity study described above were used.

3. Results and Conclusions

Serum concentrations of IgE in control rats and those fed 0.25% of 2,6-DNT for various periods are shown in Table 58. 2,6-DNT did not increase IgE concentrations.

D. Summary

The 2,6-DNT intake of the males fed the low, middle or high level of 2,6-DNT in the feed averaged 7, 35 and 145 mg/kg/day, respectively. The 2,6-DNT intake of the females averaged 7, 37 and 155 mg/kg/day, respectively. The low level produced no unequivocal effects. The middle level decreased feed consumption and weight gain and increased SGPT in some rats. There were extramedullary hematopoiesis in the spleen and/or liver, bile duct hyperplasia, and/or depression of spermatogenesis and testicular atrophy. The high level also produced methemoglobinemia, Heinz bodies, anemia and compensatory reticulocytosis. The effects in high dosage rats were more severe and occurred earlier. As for dogs, there was partial recovery in rats 4 weeks after the treatment was discontinued. The middle level of 2,6-DNT in the feed (about 35 mg/kg/day) for 6 weeks increased the number of chromatid breaks in lymphocyte culture. Continuing the feeding for 13 weeks also caused an increase in tetraploids in kidney cultures. 2,6-DNT was not mutagenic in the specific locus mutation test using Chinese hamster ovary cultures and did not affect the serum immunoglobin E titers.

TABLE 20

BODY WEIGHTS OF MALE RATS FED 2,6-DNT

% 2.6-DNT in Feed	Body Weights (gm) [a]				
	Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	216±4	435±23			
0.01	208±4	399±6			
0.05	223±3	328±9			
0.25	231±4	257±7			
0	236±5	422±6 [b]	511±3		
0.01	225±9	330±32 [b]	515±40		
0.05	228±3	358±22 [b]	419±14		
0.25	225±2	282±7 [b]	405±17		
0	221±7	417±13	518±21	570±23	
0.01	219±5	424±13	478±21	503±34	
0.05	216±7	335±10	372±8	451±13	
0.25	221±4	253±8	266±4	255±7	
0	219±6	395±10	481±21	550±20 [b]	569±21
0.01	218±5	385±14	470±15	498±11 [b]	549±16
0.05	209±9	326±6	371±9	411±14 [b]	453±7
0.25	230±5	244±8	275±4	263±8 [b]	335±11

a/ Mean ± S.E. of four rats.

b/ 2.6-DNT in feed discontinued thereafter.

TABLE 21

BODY WEIGHTS OF FEMALE RATS FED 2,6-DNT

% 2,6-DNT in Feed	Body Weights (gm) [a]				
	Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	252±1	254±1			
0.01	165±9	231±7			
0.05	166±5	210±9			
0.25	183±12	183±12			
0	168±4	268±22 [b]	279±7		
0.01	163±4	249±6 [b]	291±5		
0.05	173±4	230±5 [b]	258±8		
0.25	183±5	176±11 [b]	241±11		
0	166±4	249±6	283±10	301±10	
0.01	165±8	232±10	266±7	275±7	
0.05	167±2	223±3	257±3	214±17	
0.25	172±2	186±7	198±10	184±12	
0	181±5	248±3	285±4	305±4 [b]	332±8
0.01	176±4	235±8	266±10	282±7 [b]	307±8
0.05	178±5	208±5	236±7	251±6 [b]	266±8
0.25	180±9	163±12	177±12	222±27 [b]	201±5

[a] Mean ± S.E. of four rats.
[b] 2,6-DNT in feed discontinued thereafter.

TABLE 22

AVERAGE FEED CONSUMPTION (gm/rat/day) OF RATS FED 2,6-DNT

% 2,6-DNT in Feed	Males				
	1-4 [a]	5-8	5-8 [b]	9-13	14-17 [b]
0	26.3	28.4	28.9	33.0	30.6
0.01	25.4	27.0	27.7	35.2	25.5
0.05	20.6	20.4	23.7	30.7	28.8
0.25	12.3	15.2	24.7	16.5	24.4
	Females				
	1-4	5-8	5-8 [b]	9-13	14-17 [b]
0	21.5	18.6	19.0	23.9	21.7
0.01	15.6	16.6	18.7	21.8	20.2
0.05	14.3	14.3	16.2	21.2	16.7
0.25	8.4	10.5	16.3	16.1	10.8

a/ In weeks.
b/ Recovery period after 2,6-DNT in feed discontinued.

TABLE 23

AVERAGE 2,6-DNT INTAKE (mg/kg/day) OF RATS DURING TREATMENT

% 2,6-DNT in Feed	Males			
	1-4 [a]	5-8	9-13	1-13
0.01	8.5	6.2	7.2	7.2
0.05	37.0	29.0	38.1	35.1
0.25	126.0	145.5	158.0	144.7
	Females			
	1-4	5-8	9-13	1-13
0.01	7.7	6.7	8.1	7.4
0.05	37.6	30.9	44.1	37.1
0.25	116.8	144.5	205.5	155.0

[a] In weeks.

TABLE 24

HEMATOLOGY DATA OF CONTROL MALE RATS FOR 2,6-DNT

(B.N) BASELINE
(C.N) CONTROL
N = NUMBER OF RATS

	WKS 0 (B. 4)	WKS 4 (C. 4)	WKS 8 (C. 4)	WKS 13 (C. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.63 ± .24	7.15 ± .17	6.66 ± .07	6.81 ± .20
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	3.12 ± .34	1.39 ± .23[a/]	1.37 ± .23[a/]	1.00 ± .12[a/]
HEMATOCRIT, VOL. %	49.3 ± 1.5	50.8 ± .9	52.0 ± 1.4	50.8 ± .9
HEMOGLOBIN, GM. %	15.9 ± .5	16.4 ± .2	16.5 ± .4	16.5 ± .4
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.6 ± .6
MCV, CUBIC MICRONS	74.5 ± 2.5	71.1 ± 2.0	78.0 ± 1.5	74.6 ± 1.6
MCHH, MICRO MICROGMS.	24.0 ± .9	23.0 ± .7	24.8 ± .4	24.3 ± .6
MCHRC, GM %	32.3 ± .1	32.3 ± .3	31.8 ± .4	32.6 ± .4
PLATELETS ($X10^5$ /MM^3)	4.2 ± .2	7.6 ± .4[a/]	6.6 ± .2[a/]	5.5 ± .2[a/]
LEUKOCYTES ($X10^3$ /MM^3)	18.1 ± .7	17.4 ± 1.7	15.6 ± .7	20.7 ± 1.9
NEUTROPHILS, %	8.5 ± 2.7	13.0 ± 2.7	13.3 ± 2.3	18.3 ± 6.3
LYMPHOCYTES, %	89.8 ± 2.5	85.3 ± 2.8	83.3 ± 2.6	80.3 ± 6.5
BANDS, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.5 ± .3	0.0 ± 0.0[a/]	.8 ± .3	.3 ± .3[a/]
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	1.8 ± 1.0	2.8 ± 1.0	1.3 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from baseline (Dunnett's multiple comparison procedure[4/]).

TABLE 25

HEMATOLOGY DATA OF MALE RATS FED 2,6-DNT

DOSE 0.01 % IN FEED

(B,N) BASELINE
(T,N) TREATMENT
N = NUMBER OF RATS

	WKS 0 (B, 4)	WKS 4 (T, 4)	WKS 8 (T, 4)	WKS 13 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.39 ± .11	6.94 ± .03	6.18 ± .23	6.88 ± .57
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	3.23 ± .33	1.73 ± .13[a/]	1.86 ± .16[a/]	.85 ± .20[a/]
HEMATOCRIT, VOL, %	48.0 ± 1.2	49.5 ± .6	47.8 ± 1.0[b/]	50.3 ± .9
HEMOGLOBIN, GM, %	15.5 ± .4	15.9 ± .2	15.6 ± .3	16.2 ± .1
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	75.2 ± 1.0	71.3 ± 1.0	77.5 ± 2.1	75.2 ± 8.4
MCHH, MICRO MICROGMS,	24.3 ± .3	22.9 ± .4	25.3 ± .8	24.1 ± 2.4
MCHHC, GM %	32.3 ± .5	32.2 ± .2	32.6 ± .4	32.3 ± .5
PLATELETS ($X10^5$ /MM^3)	4.7 ± .6	6.2 ± .5	7.1 ± .4[a/]	7.3 ± .5[a/]
LEUKOCYTES ($X10^3$ /MM^3)	17.5 ± 1.0	19.5 ± 1.1	21.0 ± 1.5	25.2 ± 4.9
NEUTROPHILS, %	14.8 ± 1.9	9.3 ± 1.6	10.3 ± 1.6	23.5 ± 10.3
LYMPHOCYTES, %	84.0 ± 2.3	89.3 ± 2.0	87.8 ± 2.2	73.5 ± 12.3
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
EOSINOPHILS, %	.5 ± .5	.3 ± .3	.3 ± .3	.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.8 ± .5	1.3 ± .6	1.8 ± .8	2.5 ± 1.8
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4/]).
b/ Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[4/]).

TABLE 26

HEMATOLOGY DATA OF MALE RATS FED 2,6-DNT

DOSE .05 % IN FEED

(B.N) BASELINE
(T.N) TREATMENT
N = NUMBER OF RATS

	WKS 0 (B. 4)	WKS 4 (T. 4)	WKS 8 (T. 4)	WKS 13 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.86 ± .20	7.78 ± .18[a/]	6.51 ± .10	7.76 ± .15[a/]
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	3.15 ± .57	.85 ± .09[a/]	1.74 ± .15[a/]	.81 ± .16[a/]
HEMATOCRIT, VOL, %	46.0 ± 2.2	52.5 ± .5[a/]	49.0 ± .4	52.0 ± 1.3[a/]
HEMOGLOBIN, GM, %	15.6 ± .6	16.8 ± .3	15.9 ± .2	16.9 ± .2
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	66.9 ± 1.4[b/]	67.6 ± 1.4	75.3 ± 1.8[a/]	67.1 ± 1.7
MCHB, MICRO MICROGMS,	22.7 ± .2	21.6 ± .1	24.5 ± .6[a/]	21.8 ± .5
MCHBC, GM %	33.9 ± .3	32.0 ± .5	32.6 ± .1	32.6 ± .9
PLATELETS ($X10^5$ /MM^3)	5.7 ± .2	7.8 ± .2	8.7 ± .8[a/]	8.8 ± .8[a,b/]
LEUKOCYTES ($X10^3$ /MM^3)	16.6 ± 1.3	22.2 ± 1.2	21.5 ± 1.9	27.4 ± 3.1[a/]
NEUTROPHILS, %	11.0 ± 1.5	10.8 ± 2.2	14.8 ± 2.0	23.3 ± 2.4[a/]
LYMPHOCYTES, %	88.5 ± 1.6	88.0 ± 1.7	81.5 ± 2.8	76.0 ± 3.1[a/]
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.3 ± .3	.3 ± .3	1.5 ± .6	.5 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	1.0 ± .4	2.3 ± 1.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4/]).
b/ Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[4/]).

TABLE 27

HEMATOLOGY DATA OF MALE RATS FED 2,6-DNT

DOSE 0.25 % IN FEED

(B,N) BASELINE
(T,N) TREATMENT
N = NUMBER OF RATS

	WKS 0 (B, 4)	WKS 4 (T, 4)	WKS 8 (T, 4)	WKS 13 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.29 ± .19	5.80 ± .35[b/]	5.68 ± .19[b/]	7.40 ± .14[a/]
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	3.23 ± .59	9.33 ± 1.27[a,b/]	3.94 ± .56[b/]	.82 ± .26
HEMATOCRIT, VOL, %	46.0 ± 1.6	45.8 ± 1.5[b/]	48.0 ± 1.5	52.5 ± 2.3
HEMOGLOBIN, GM, %	15.2 ± .3	14.3 ± .5[b/]	14.7 ± .8	17.1 ± .5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	.6 ± .6	3.0 ± 1.2[a/]
MCV, CUBIC MICRONS	73.1 ± 1.3	79.3 ± 3.1[b/]	81.9 ± 2.7	70.9 ± 2.2
MCHA, MICRO MICROGMS.	24.2 ± .6	24.8 ± .7	25.8 ± .9	23.1 ± .4
MCHBC, GM %	33.2 ± .6	31.3 ± .6	32.0 ± .6	32.6 ± .5
PLATELETS ($X10^5$ /MM^3)	4.9 ± .4	9.5 ± .9[a/]	9.8 ± .8[a,b/]	7.6 ± .8
LEUKOCYTES ($X10^3$ /MM^3)	16.2 ± .9	31.5 ± 2.0[a,b/]	27.8 ± 2.5[a,b/]	25.0 ± 4.0
NEUTROPHILS, %	12.0 ± 1.4	10.3 ± 4.5	8.5 ± 1.4	20.3 ± 3.0
LYMPHOCYTES, %	87.0 ± 1.4	88.5 ± 4.9	90.0 ± 1.5	76.5 ± 3.2
BANDS, %	.3 ± .3	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.3 ± .3	.5 ± .3	.3 ± .3	1.8 ± .5[a/]
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .3	.5 ± .3	1.3 ± .3	1.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4/]).
b/ Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[4/]).

TABLE 28

HEMATOLOGY DATA OF MALE RATS FED 2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C, N) CONTROL
(T,N) TREATMENT
N = NUMBER OF RATS

Dose (% in feed):	0.0 (C,4)	0.25 (T,4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.66 ± .07	6.06 ± .14[a/]
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.37 ± .23	.96 ± .14
HEMATOCRIT, VOL. %	52.0 ± 1.4	47.0 ± 1.0[a/]
HEMOGLOBIN, GM. %	16.5 ± .4	15.6 ± .3
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	78.0 ± 1.5	77.6 ± .2
MCHB, MICRO MICROGMS.	24.8 ± .4	25.8 ± .1
MCHBC, GM %	31.8 ± .4	33.2 ± .1[a/]
PLATELETS ($X10^5$ /MM^3)	6.6 ± .2	4.6 ± .2[a/]
LEUKOCYTES ($X10^3$ /MM^3)	15.6 ± .7	10.5 ± 1.5[a/]
NEUTROPHILS, %	13.3 ± 2.3	7.5 ± 1.8
LYMPHOCYTES, %	83.3 ± 2.6	91.0 ± 1.8
BANDS, %	0.0 ± 0.0	.3 ± .3
EOSINOPHILS, %	.8 ± .3	.8 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	2.8 ± 1.0	.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control (Dunnett's multiple comparison procedure[4/]).

TABLE 29

HEMATOLOGY DATA OF MALE RATS FED 2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C,N) CONTROL
(T.N) TREATMENT
N = NUMBER OF RATS

Dose (% in feed):	0.0 (C, 4)	0.01 (T, 4)	0.05 (T, 4)	0.25 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.70 ± .08	7.75 ± .07	7.57 ± .20	7.26 ± .24
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.05 ± .24	1.07 ± .40	1.59 ± .67	1.19 ± .23
HEMATOCRIT, VOL. %	48.0 ± .7	45.5 ± 1.2	45.5 ± 3.5	46.5 ± 1.0
HEMOGLOBIN, GM. %	16.2 ± .2	16.4 ± .3	15.9 ± .5	15.9 ± .2
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	62.3 ± 1.1	58.7 ± 1.4	59.9 ± 3.3	64.4 ± 3.6
MCHH, MICRO MICROGMS.	21.1 ± .1	21.2 ± .4	21.1 ± .3	22.1 ± 1.0
MCHHC, GM %	33.8 ± .7	36.1 ± .6	35.5 ± 1.9	34.3 ± .4
PLATELETS ($X10^5$ /MM^3)	4.9 ± .3	4.9 ± .3	3.2 ± .7	5.5 ± .5
LEUKOCYTES ($X10^3$ /MM^3)	8.3 ± 1.2	9.0 ± 1.1	9.9 ± .7	9.1 ± .8
NEUTROPHILS, %	22.0 ± 2.5	15.0 ± 1.8	12.5 ± 2.6[a/]	16.5 ± 2.4
LYMPHOCYTES, %	74.8 ± 2.4	84.3 ± 2.2	86.5 ± 2.9[a/]	82.8 ± 2.4
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.3 ± 1.3	.3 ± .3	.8 ± .5	.8 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.0 ± .4	.5 ± .5	.3 ± .3	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control (Dunnett's multiple comparison procedure[4/]).

TABLE 30

LABORATORY DATA OF MALE RATS FED 2,6-DNT
FOR 4 WEEKS

(C, N) CONTROL
(T,N) TREATMENT
N = NUMBER OF RATS

Dose (% in feed):	0.0 (C, 4)	0.01 (T, 4)	0.05 (T, 4)	0.25 (T, 4)
GLUCOSE (FASTING), MG %	86.8 ± 11.2	93.8 ± 5.8	113.3 ± 9.5	73.3 ± 8.8
SGOT, IU/L	131 ± 20	142 ± 18	136 ± 17	160 ± 20
SGPT, IU/L	42.3 ± 4.8	40.8 ± 2.8	56.5 ± 1.9	65.5 ± 6.2 a/
ALK. PHOS., IU/L	111 ± 6	125 ± 6	109 ± 6	87 ± 9
BUN, MG %	14.3 ± .5	15.5 ± .6	15.3 ± 1.4	13.0 ± .9
MG (MEQ/L)	2.5 ± .2	2.4 ± .1	2.6 ± .1	2.9 ± .1
CA (MEQ/L)	5.1 ± .1	5.0 ± .0	4.9 ± .1	4.9 ± .2
NA (MEQ/L)	146 ± 2	143 ± 1	144 ± 1	146 ± 2
K (MEQ/L)	5.2 ± .1	5.2 ± .1	5.8 ± .3	5.4 ± .5
CL (MEQ/L)	98 ± 1	97 ± 1	100 ± 1	103 ± 1 a/

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4]).

TABLE 31

LABORATORY DATA OF MALE RATS FED 2,6-DNT FOR 13 WEEKS

(C,N) CONTROL
(T,N) TREATMENT
N = NUMBER OF RATS

Dose (% in feed):	0.0 (C, 4)	0.01 (T, 4)	0.05 (T, 4)	0.25 (T, 4)
GLUCOSE (FASTING), MG %	127.0 ± 9.6	133.8 ± 3.1	121.5 ± 8.8	111.0 ± 7.7
SGOT, IU/L	146 ± 5	112 ± 8	101 ± 9[a/]	133 ± 14
SGPT, IU/L	45.3 ± 2.3	40.0 ± 2.1	58.5 ± 2.4	51.5 ± 7.0
ALK. PHOS., IU/L	57 ± 3	56 ± 5	52 ± 4	122 ± 22[a/]
BUN, MG %	16.8 ± .8	15.5 ± .5	20.5 ± 1.9	23.5 ± 1.9[a/]
MG (MEQ/L)	2.0 ± .1	2.1 ± .0	1.7 ± .1[a/]	2.2 ± .1
CA (MEQ/L)	4.7 ± .1	4.9 ± .0	4.8 ± .1	4.7 ± .1
NA (MEQ/L)	140 ± 1	140 ± 1	140 ± 0	138 ± 1
K (MEQ/L)	6.3 ± .3	6.7 ± .4	5.9 ± .1	6.8 ± .2
CL (MEQ/L)	103 ± 1	100 ± 1	104 ± 2	105 ± 1

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4/]).

TABLE 32

LABORATORY DATA OF MALE RATS FED 2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C,N) CONTROL
(T,N) TREATMENT
N = NUMBER OF RATS

Dose (% in feed):	0.0 (C, 4)	0.01 (T, 4)	0.05 (T, 4)	0.25 (T, 4)
GLUCOSE (FASTING), MG %	103.8 ± 6.6	109.5 ± 3.5	96.0 ± 3.7	93.8 ± 2.2
SGOT, IU/L	105 ± 8	87 ± 5	106 ± 8	133 ± 19
SGPT, IU/L	41.5 ± 4.0	32.5 ± .9	55.3 ± 4.6[a/]	39.3 ± 1.4
ALK. PHOS., IU/L	87 ± 6	81 ± 8	103 ± 14	93 ± 4
BUN, MG %	21.3 ± 1.3	19.3 ± 1.0	20.5 ± 1.3	18.8 ± 1.7
MG (MEQ/L)	2.2 ± .1	2.0 ± .1	2.0 ± .1	2.3 ± .0
CA (MEQ/L)	5.1 ± .1	5.1 ± .0	4.9 ± .0	5.1 ± .0
NA (MEQ/L)	148 ± 1	146 ± 2	142 ± 1[a/]	143 ± 0[a/]
K (MEQ/L)	4.8 ± .2	5.0 ± .2	5.0 ± .1	4.9 ± .1
CL (MEQ/L)	107 ± 2	106 ± 1	107 ± 1	105 ± 0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4/]).

TABLE 33

LABORATORY DATA OF MALE RATS FED 2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C,N) CONTROL
(T,N) TREATMENT
N = NUMBER OF RATS

Dose (% in feed):	0.0 (C, 4)	0.01 (T, 4)	0.05 (T, 4)	0.25 (T, 4)
GLUCOSE (FASTING), MG %	78.3 ± 11.6	89.0 ± 5.6	109.5 ± 7.6	84.5 ± 11.3
SGOT, IU/L	134 ± 23	132 ± 15	142 ± 29	151 ± 19
SGPT, IU/L	38.5 ± 4.7	42.3 ± 3.3	43.0 ± 4.2	52.3 ± 8.4
ALK. PHOS., IU/L	60 ± 3	62 ± 6	71 ± 10	103 ± 7 a/
BUN, MG %	15.5 ± 1.0	18.0 ± 1.3	20.3 ± .8 a/	26.5 ± 1.7 a/
MG (MEQ/L)	1.9 ± .1	1.9 ± .0	1.8 ± .1	1.9 ± .0
CA (MEQ/L)	4.7 ± .0	4.8 ± .0	5.0 ± .1 a/	5.5 ± .0 a/
NA (MEQ/L)	140 ± 1	139 ± 1	141 ± 0	141 ± 1
K (MEQ/L)	5.0 ± .1	5.4 ± .2	5.4 ± .4	5.0 ± .3
CL (MEQ/L)	101 ± 0	102 ± 2	104 ± 1	98 ± 1

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4]).

TABLE 34

HEMATOLOGY DATA OF FEMALE CONTROL RATS FOR 2,6-DNT

(B.N) BASELINE
(C.N) CONTROL
N = NUMBER OF RATS

	WKS 0 (B. 4)	WKS 4 (C. 4)	WKS 8 (C. 4)	WKS 13 (C. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.39 ± .21	6.92 ± .18	5.32 ± .33	6.49 ± .44
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	2.61 ± .92	1.18 ± .20	1.51 ± .18	1.38 ± .16
HEMATOCRIT, VOL. %	46.5 ± 1.0	50.8 ± 1.8	51.0 ± .9	50.8 ± .8
HEMOGLOBIN, GM. %	14.9 ± .4	16.8 ± .5 a/	17.0 ± .4 a/	16.5 ± .6
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.6 ± .6
MCV, CUBIC MICRONS	72.9 ± 1.2	73.3 ± 1.9	97.0 ± 6.3 a/	79.2 ± 5.2
MCMB, MICRO MICROGMS.	23.4 ± .2	24.4 ± .5	32.2 ± 1.8 a/	25.6 ± 1.4
MCHBC, GM %	32.1 ± .3	33.2 ± .3	33.3 ± .5	32.4 ± .8
PLATELETS ($X10^5$ /MM^3)	5.9 ± .3	7.0 ± 1.2	5.6 ± .5	6.5 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	15.3 ± .8	19.3 ± 1.7	14.6 ± 1.5	17.5 ± 2.7
NEUTROPHILS, %	12.3 ± 1.4	11.5 ± 1.3	13.3 ± 1.5	21.5 ± 5.8
LYMPHOCYTES, %	84.8 ± 2.3	86.0 ± 2.3	82.5 ± 2.1	77.0 ± 5.8
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.5 ± .3	2.0 ± .9	1.5 ± .3	1.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.5 ± .6	.5 ± .3	2.8 ± 1.1	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from baseline (Dunnett's multiple comparison procedure[4]).

TABLE 35

HEMATOLOGY DATA OF FEMALE RATS FED 2,6-DNT

DOSE 0.01 % IN FEED

(B.N) BASELINE
(T.N) TREATMENT
N = NUMBER OF RATS

	WKS 0 (B. 4)	WKS 4 (T. 4)	WKS 8 (T. 4)	WKS 13 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.42 ± .04	6.35 ± .31	4.86 ± .23[a/]	6.35 ± .28
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	3.55 ± .52	1.07 ± .15[a/]	2.01 ± .30[a/]	1.24 ± .40[a/]
HEMATOCRIT, VOL, %	44.3 ± .5	47.5 ± .5[a/]	50.3 ± 1.0[a/]	47.8 ± .9[a/]
HEMOGLOBIN, GM, %	15.0 ± .4	15.8 ± .3	16.3 ± .4	15.7 ± .4
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.0
MCV, CUBIC MICRONS	68.9 ± .8[b/]	75.3 ± 3.4	104.1 ± 5.9[a/]	75.5 ± 2.7
MCHB, MICRO MICROGMS,	23.4 ± .8	25.1 ± 1.0	33.8 ± 1.9[a/]	24.8 ± .8
MCHHC, GM %	34.0 ± .8[b/]	33.4 ± .3	32.4 ± .2	32.8 ± .2
PLATELETS ($X10^5$ /MM^3)	6.6 ± .3	7.3 ± .9	8.1 ± .7	8.5 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	16.6 ± 2.0	17.6 ± 1.6	23.0 ± 1.0	20.1 ± 2.0
NEUTROPHILS, %	10.3 ± 1.1	14.8 ± 3.4	10.5 ± 2.6	18.8 ± 5.3
LYMPHOCYTES, %	88.3 ± .9	81.5 ± 4.5	85.8 ± 2.8	79.5 ± 5.5
BANDS, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0[b/]	2.0 ± 1.1	.5 ± .3	1.3 ± .9
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.5 ± .3	1.5 ± .5	3.3 ± 1.0	.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4/]).
b/ Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[4/]).

TABLE 36

HEMATOLOGY DATA OF FEMALE RATS FED 2,6-DNT

DOSE 0.05 % IN FEED

(B.N) BASELINE
(T.N) TREATMENT
N = NUMBER OF RATS

	WKS 0 (B. 4)	WKS 4 (T. 4)	WKS 8 (T. 4)	WKS 13 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.66 ± .10	6.78 ± .20	5.94 ± .44	6.44 ± .24
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	3.36 ± .29	.85 ± .26[a/]	1.45 ± .21[a/]	1.13 ± .15[a/]
HEMATOCRIT, VOL. %	45.8 ± .8	48.0 ± 1.4	50.0 ± .6[a/]	51.0 ± .9[a/]
HEMOGLOBIN, GM. %	15.1 ± .3	15.8 ± .5	16.3 ± .6	16.1 ± .4
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	68.7 ± .8[b/]	70.9 ± 1.9	85.9 ± 7.9	79.5 ± 3.6
MCHH, MICRO MICROGMS.	22.7 ± .3	23.4 ± .6	27.8 ± 1.6[a/]	25.1 ± 1.0
MCHBC, GM %	33.1 ± .2	33.0 ± .1	32.7 ± 1.5	31.6 ± .5
PLATELETS ($X10^5$ /MM^3)	5.2 ± .3	7.2 ± .3	7.3 ± .3	8.9 ± 1.6[a/]
LEUKOCYTES ($X10^3$ /MM^3)	16.7 ± 2.2	22.7 ± 2.1	24.2 ± 2.5[b/]	26.4 ± 2.7[a/]
NEUTROPHILS, %	9.5 ± 1.6	14.8 ± 3.5	10.8 ± 1.1	13.8 ± 1.9
LYMPHOCYTES, %	88.0 ± 1.4	82.5 ± 3.5	86.3 ± 2.4	85.5 ± 1.7
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.3 ± .3[b/]	1.0 ± .4	0.0 ± 0.0[b/]	.8 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	2.3 ± .3	1.8 ± .5	3.0 ± 1.5	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4/]).
b/ Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[4/]).

TABLE 37

HEMATOLOGY DATA OF FEMALE RATS FED 2,6-DNT

DOSE 0.25 % IN FEED

(B,N) BASELINE
(T,N) TREATMENT
N = NUMBER OF RATS

	WKS 0 (B, 4)	WKS 4 (T, 4)	WKS 8 (T, 4)	WKS 13 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.81 ± .10	6.91 ± .22	5.76 ± .24[a/]	7.06 ± .26
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	2.33 ± .41	5.24 ± 1.12[b/]	4.06 ± .95[b/]	.89 ± .10
HEMATOCRIT, VOL, %	47.3 ± .5	53.5 ± 2.0[a/]	52.3 ± .7	53.3 ± 1.3[a/]
HEMOGLOBIN, GM, %	15.5 ± .1	16.9 ± .8	16.1 ± .3	16.8 ± .2
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	.6 ± .6	2.4 ± 1.0[a/]
MCV, CUBIC MICRONS	69.4 ± 1.0	77.5 ± 2.1	90.8 ± 4.3[a/]	75.9 ± 4.3
MCHR, MICRO MICROGMS,	22.9 ± .5	24.5 ± 1.0	28.1 ± .7[a/]	24.0 ± .8
MCHBC, GM %	32.9 ± .4	31.5 ± .5[b/]	31.1 ± .4	31.7 ± .7
PLATELETS ($X10^5$ /MM^3)	5.8 ± .7	9.1 ± 1.0	8.9 ± 1.1[b/]	8.8 ± 1.3
LEUKOCYTES ($X10^3$ /MM^3)	14.3 ± 1.4	24.0 ± 3.1[a/]	24.2 ± 3.3[a,b/]	20.5 ± 1.7
NEUTROPHILS, %	10.3 ± 1.9	10.8 ± 3.1	10.3 ± 2.0	21.0 ± 3.7[a/]
LYMPHOCYTES, %	87.5 ± 1.6	86.8 ± 2.8	87.3 ± 2.6	78.5 ± 4.0
BANDS, %	.3 ± .3	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .5	1.0 ± .6	1.0 ± .6	0.0 ± 0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.3 ± .6	1.3 ± .9	1.5 ± .9	.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4/]).
b/ Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[4/]).

TABLE 38

HEMATOLOGY DATA OF FEMALE RATS FED 2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C, N) CONTROL
(T,N) TREATMENT
N = NUMBER OF RATS

Dose (% in feed):	0.0 (C, 4)	0.25 (T, 3)
ERYTHROCYTES ($X10^6$ /MM^3)	5.32 ± .33	5.57 ± .19
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.51 ± .18	.88 ± .06[a/]
HEMATOCRIT, VOL, %	51.0 ± .9	46.7 ± .9[a/]
HEMOGLOBIN, GM, %	17.0 ± .4	15.3 ± .4[a/]
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	97.0 ± 6.3	84.1 ± 3.6
MCHB, MICRO MICROGMS.	32.2 ± 1.8	27.5 ± 1.5
MCHBC, GM %	33.3 ± .5	32.7 ± .3
PLATELETS ($X10^5$ /MM^3)	5.6 ± .5	5.1 ± 1.1
LEUKOCYTES ($X10^3$ /MM^3)	14.6 ± 1.5	8.4 ± .4[a/]
NEUTROPHILS, %	13.3 ± 1.5	8.0 ± 1.5
LYMPHOCYTES, %	82.5 ± 2.1	90.7 ± 2.3[a/]
BANDS, %	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.5 ± .3	1.3 ± .9
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	2.8 ± 1.1	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control (Dunnett's multiple comparison procedure[4/]).

TABLE 39

HEMATOLOGY DATA OF FEMALE RATS FED 2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C,N) Control
(T,N) TREATMENT
N = NUMBER OF RATS

Dose (% in feed):	0.0 (C,4)	0.01 (T,4)	0.05 (T,4)	0.25 (T,4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.23 ± .22	6.83 ± .29	6.39 ± .23	7.21 ± .11
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.03 ± .11	1.41 ± .78	1.62 ± 1.21	.61 ± .17
HEMATOCRIT, VOL, %	47.0 ± .4	44.8 ± 1.5	43.5 ± 2.2	47.0 ± .4
HEMOGLOBIN, GM, %	16.3 ± .1	15.1 ± .5	14.9 ± .8	16.3 ± .2
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	65.2 ± 2.2	65.6 ± .9	68.0 ± 1.9	65.3 ± 1.4
MCHB, MICRO MICROGMS.	22.6 ± .6	22.2 ± .3	23.4 ± .6	22.6 ± .5
MCHBC, GM %	34.6 ± .3	33.8 ± .3	34.4 ± .4	34.6 ± .4
PLATELETS ($X10^5$ /MM^3)	4.5 ± .3	4.8 ± .8	5.4 ± .4	4.3 ± .5
LEUKOCYTES ($X10^3$ /MM^3)	6.1 ± .4	6.5 ± .8	7.4 ± .9	6.8 ± .4
NEUTROPHILS, %	18.3 ± 2.6	14.0 ± 2.6	15.5 ± 5.5	6.3 ± 1.0
LYMPHOCYTES, %	78.3 ± 2.4	83.0 ± 2.5	83.5 ± 6.1	92.8 ± 1.3[a/]
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.5 ± 1.4	2.3 ± 1.1	.5 ± .5	.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.0 ± .4	.8 ± .5	.5 ± .3	.8 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control (Dunnett's multiple comparison procedure[4/]).

TABLE 40

LABORATORY DATA OF FEMALE RATS FED 2,6-DNT FOR 4 WEEKS

(C,N) Control
(T,N) TREATMENT
N = NUMBER OF RATS

Dose (% in feed):	0.0 (C, 4)	0.01 (T, 4)	0.05 (T, 4)	0.25 (T, 4)
GLUCOSE (FASTING), MG %	103.0 ± 17.9	113.8 ± 7.9	111.3 ± 6.5	90.3 ± 14.6
SGOT, IU/L	183 ± 54	103 ± 7	161 ± 24	214 ± 15
SGPT, IU/L	39.3 ± 5.4	41.5 ± 2.9	52.3 ± 6.4	69.3 ± 9.4[a/]
ALK. PHOS., IU/L	90 ± 7	69 ± 7	70 ± 3	70 ± 11
BUN, MG %	13.8 ± 1.1	14.5 ± 1.3	15.0 ± 1.7	18.5 ± 2.7
MG (MEQ/L)	2.5 ± .1	2.5 ± .1	2.6 ± .2	3.4 ± .3[a/]
CA (MEQ/L)	5.1 ± .0	5.1 ± .1	5.3 ± .1	5.1 ± .3
NA (MEQ/L)	142 ± 0	143 ± 1	145 ± 1	146 ± 1
K (MEQ/L)	5.4 ± .4	5.8 ± .3	6.9 ± .5	6.8 ± .9
CL (MEQ/L)	98 ± 2	103 ± 1	104 ± 2	105 ± 2

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4/]).

TABLE 41

LABORATORY DATA OF FEMALE RATS FED 2,6-DNT FOR 13 WEEKS

(C,N) Control
(T,N) TREATMENT
N = NUMBER OF RATS

Dose (% in feed):	0.0 (C, 4)	0.01 (T, 4)	0.05 (T, 4)	0.25 (T, 4)
GLUCOSE (FASTING), MG %	121.5 ± 8.5	114.8 ± 2.9	116.8 ± 10.1	110.3 ± 6.8
SGOT, IU/L	85 ± 4	121 ± 17	84 ± 7	167 ± 38
SGPT, IU/L	32.5 ± .9	32.5 ± 1.9	35.5 ± 1.9	49.3 ± 8.5
ALK. PHOS., IU/L	38 ± 2	43 ± 7	33 ± 3	75 ± 3[a/]
BUN, MG %	21.0 ± 1.9	19.8 ± 1.8	21.8 ± 5.3	26.3 ± 2.3
MG (MEQ/L)	2.0 ± .1	2.1 ± .1	2.0 ± .1	2.4 ± .1[a/]
CA (MEQ/L)	5.0 ± .1	5.0 ± 0.0	5.2 ± .1	4.6 ± .1
NA (MEQ/L)	138 ± 1	141 ± 0	142 ± 1[a/]	140 ± 1
K (MEQ/L)	6.4 ± .1	6.7 ± .2	6.4 ± .3	6.5 ± .4
CL (MEQ/L)	103 ± 1	103 ± 1	106 ± 1	105 ± 2

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4/]).

TABLE 42

LABORATORY DATA OF FEMALE RATS FED 2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C,N) Control
(T,N) TREATMENT
N = NUMBER OF RATS

Dose (% in feed):	0.0 (C, 3)	0.01 (T, 4)	0.05 (T, 4)	0.25 (T, 4)
GLUCOSE (FASTING), MG %	97.7 ± 1.9	97.8 ± 6.7	98.8 ± 5.1	99.0 ± 6.4
SGOT, IU/L	136 ± 9	178 ± 43	103 ± 8	121 ± 2
SGPT, IU/L	39.0 ± 3.6	40.0 ± 4.7	34.8 ± .8	39.3 ± 2.3
ALK. PHOS., IU/L	72 ± 6	61 ± 3	58 ± 5	76 ± 8
BUN, MG %	17.3 ± 1.5	20.5 ± 1.8	17.8 ± 1.1	22.5 ± 1.6
MG (MEQ/L)	2.0 ± .1	2.1 ± .1	2.0 ± .1	2.1 ± .1
CA (MEQ/L)	5.0 ± .1	5.1 ± .1	5.0 ± .1	4.9 ± .0
NA (MEQ/L)	141 ± 2	140 ± 1	140 ± 2	136 ± 2
K (MEQ/L)	5.0 ± .1	5.5 ± .4	4.8 ± .2	4.8 ± .1
CL (MEQ/L)	106 ± 2	105 ± 2	107 ± 1	108 ± 2

ENTRIES ARE MEAN ± STANDARD ERROR

TABLE 43

LABORATORY DATA OF FEMALE RATS FED 2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C,N) Control
(T,N) TREATMENT
N = NUMBER OF RATS

Dose (% in feed):	0.0 (C, 4)	0.01 (T, 4)	0.05 (T, 4)	0.25 (T, 4)
GLUCOSE (FASTING), MG %	101.0 ± 4.1	87.0 ± 4.8	90.3 ± 6.3	101.0 ± 9.1
SGOT, IU/L	109 ± 7	102 ± 5	98 ± 5	101 ± 15
SGPT, IU/L	31.8 ± .8	30.3 ± .8	32.5 ± 2.6	34.8 ± 2.3
ALK. PHOS., IU/L	36 ± 5	42 ± 4	47 ± 6	48 ± 6
BUN, MG %	20.8 ± 2.1	21.3 ± 1.9	21.3 ± 1.8	23.5 ± 1.7
MG (MEQ/L)	2.0 ± .1	1.9 ± .1	2.0 ± .1	1.9 ± .1
CA (MEQ/L)	4.9 ± .1	5.2 ± .1	4.8 ± .2	5.1 ± .2
NA (MEQ/L)	140 ± 1	142 ± 1	142 ± 1	141 ± 0
K (MEQ/L)	4.6 ± .3	4.7 ± .0	5.0 ± .1	4.6 ± .1
CL (MEQ/L)	103 ± 1	105 ± 1	102 ± 1	104 ± 2

ENTRIES ARE MEAN ± STANDARD ERROR

TABLE 44

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED 2,6-DNT FOR 4 WEEKS

Sex	% 2,6-DNT in Feed	Body Weight (gm)	Absolute Weights (gm) Liver	Spleen	Kidneys	Heart	Brain
Male	0	391±8	12.6±0.6	0.81±0.03	3.26±0.08	1.34±0.10	2.08±0.04
	0.01	364±18	12.7±0.6	0.72±0.03	3.01±0.12	1.26±0.03	2.02±0.09
	0.05	306±9[a]	11.7±0.5	0.64±0.03	3.04±0.21	1.06±0.02[a]	2.05±0.04
	0.25	225±5[a]	9.2±0.6[a]	0.97±0.09	2.29±0.20[a]	0.79±0.05[a]	1.94±0.07
Female	0	232±5	6.9±0.3	0.51±0.04	1.78±0.04	0.92±0.08	1.88±0.05
	0.01	210±7	6.5±0.3	0.54±0.04	1.62±0.07	0.86±0.07	1.73±0.03
	0.05	194±8[a]	7.1±0.2	0.70±0.08	1.58±0.10	0.75±0.04	1.82±0.07[a]
	0.25	157±10[a]	6.6±0.6	0.46±0.02	1.44±0.10[a]	0.58±0.07[a]	1.86±0.06

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/100 gm body weight) Liver	Spleen	Kidneys	Heart	Brain
Male	0	3.2±0.1	0.21±0.01	0.83±0.01	0.34±0.02	0.53±0.02
	0.01	3.5±0.3	0.20±0.02	0.83±0.05	0.35±0.02	0.56±0.03
	0.05	3.8±0.1	0.21±0.01	0.97±0.05	0.35±0.00	0.67±0.02[a]
	0.25	4.1±0.2[a]	0.43±0.03[a]	0.02±0.08	0.35±0.02	0.86±0.02[a]
Female	0	3.0±0.1	0.22±0.02	0.77±0.03	0.40±0.04	0.81±0.01
	0.01	3.1±0.2	0.26±0.02	0.77±0.02	0.41±0.04	0.83±0.02
	0.05	3.7±0.1[a]	0.36±0.05[a]	0.81±0.04	0.39±0.03	0.94±0.04
	0.25	4.2±0.2[a]	0.29±0.02	0.91±0.03[a]	0.37±0.04	1.20±0.07[a]

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/gm brain weight) Liver	Spleen	Kidneys	Heart
Male	0	6.1±0.4	0.39±0.02	1.57±0.06	0.65±0.05
	0.01	6.4±0.5	0.36±0.03	1.50±0.11	0.63±0.04
	0.05	5.7±0.3	0.31±0.02	1.45±0.12	0.52±0.02
	0.25	4.7±0.2	0.50±0.03[a]	1.18±0.08[a]	0.41±0.03[a]
Female	0	2.7±0.1	0.27±0.02	0.95±0.04	0.49±0.05
	0.01	3.8±0.3	0.31±0.03	0.94±0.05	0.50±0.05
	0.05	3.9±0.1	0.39±0.06	0.87±0.03	0.41±0.03
	0.25	3.5±0.3	0.25±0.01	0.77±0.04[a]	0.31±0.03[a]

Entries are mean ± standard error of four rats.

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4])

TABLE 45

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED 2,6-DNT FOR 13 WEEKS

Sex	% 2,6-DNT in Feed	Body Weight (gm)	Absolute Weights (gm) Liver	Spleen	Kidneys	Heart	Brain
Male	0	545±21	15.4±0.5	0.95±0.06	3.09±0.10	1.56±0.09	2.09±0.10
	0.01	486±24	17.0±1.3	1.02±0.03	2.33±0.18	1.60±0.15	2.17±0.07
	0.05	451±13[a]	10.7±0.2[a]	0.90±0.10	3.05±0.02	1.27±0.01	2.18±0.07
	0.25	256±12[a]	8.9±0.3[a]	0.65±0.02[a]	2.11±0.07	0.99±0.08[a]	1.97±0.05
Female	0	286±11	7.9±0.2	0.58±0.04	1.65±0.15	0.94±0.03	1.88±0.06
	0.01	270±8	8.3±0.6	0.69±0.09	1.93±0.07	0.86±0.06	2.08±0.07
	0.05	214±17[a]	7.1±0.4	0.54±0.05	1.71±0.04	0.87±0.06	2.00±0.04
	0.25	176±9[a]	7.2±0.2	0.57±0.06	1.60±0.08	0.66±0.05[a]	1.86±0.06

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/100 gm body weight) Liver	Spleen	Kidneys	Heart	Brain
Male	0	2.8±0.0	0.17±0.00	0.57±0.03	0.29±0.01	0.39±0.03
	0.01	3.5±0.3[a]	0.21±0.01	0.48±0.03	0.33±0.03	0.45±0.02
	0.05	2.4±0.1	0.20±0.02	0.68±0.02	0.29±0.00	0.48±0.00
	25	3.5±0.1[a]	0.26±0.02[a]	0.83±0.06[a]	0.39±0.02[a]	0.78±0.05[a]
Female	0	2.8±0.0	0.20±0.01	0.66±0.01	0.33±0.01	0.66±0.03
	0.01	3.1±0.2	0.25±0.03	0.62±0.11	0.32±0.02	0.77±0.05
	0.05	3.4±0.3	0.26±0.04	0.81±0.06	0.41±0.03	0.95±0.06[a]
	0.25	4.1±0.1[a]	0.32±0.02[a]	0.91±0.06	0.38±0.03	1.06±0.03[a]

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/gm brain weight) Liver	Spleen	Kidneys	Heart
Male	0	7.4±0.5	0.46±0.04	1.49±0.12	0.78±0.08
	0.01	7.9±0.6	0.47±0.02	1.08±0.10[a]	0.74±0.08
	0.05	5.0±0.2[a]	0.41±0.05	1.41±0.04	0.65±0.02
	0.25	4.6±0.2[a]	0.33±0.02	1.07±0.02[a]	0.51±0.05[a]
Female	0	4.2±0.2	0.31±0.02	1.01±0.03	0.50±0.01
	0.01	4.0±0.4	0.34±0.05	0.82±0.16	0.42±0.04
	0.05	3.5±0.2	0.27±0.03	0.86±0.01	0.44±0.03
	0.25	3.9±0.1	0.31±0.02	0.87±0.07	0.36±0.04[a]

Entries are mean ± standard error of four rats.

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4])

TABLE 46

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED 2,6-DNT 4 WEEKS AND ALLOWED TO RECOVER 4 WEEKS

Sex	% 2,6-DNT in Feed	Body Weight (gm)	Absolute Weights (gm) Liver	Spleen	Kidneys	Heart	Brain
Male	0	486±4	14.8±0.3	0.85±0.04	3.34±0.10	1.48±0.05	2.24±0.10
	0.01	446±11	12.1±1.6	0.76±0.10	2.59±0.39	1.67±0.20	2.26±0.18
	0.05	389±16[a]	12.8±1.0	0.98±0.21	2.21±0.08[a]	1.45±0.11	2.13±0.11
	0.25	403±5[a]	13.6±1.3	0.78±0.05	2.85±0.12	1.52±0.15	2.13±0.18
Female	0	256±12	6.8±0.2	0.58±0.02	1.62±0.10	1.02±0.11	1.83±0.17
	0.01	274±8	7.3±0.3	0.58±0.02	1.28±0.03[a]	0.92±0.05	1.78±0.04
	0.05	247±6	9.0±1.6	0.58±0.02	1.28±0.03[a]	1.04±0.12	2.33±0.20
	0.25	241±4	7.7±0.3	0.51±0.03	1.39±0.06[a]	1.13±0.25	2.29±0.16

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/100 gm body weight) Liver	Spleen	Kidneys	Heart	Brain
Male	0	3.1±0.1	0.18±0.01	0.69±0.02	0.31±0.01	0.46±0.02
	0.01	2.7±0.4	0.17±0.03	0.58±0.09	0.38±0.04	0.51±0.03
	0.05	3.3±0.3	0.26±0.07	0.62±0.07	0.38±0.03	0.55±0.03
	0.25	3.4±0.2	0.19±0.01	0.70±0.05	0.38±0.04	0.53±0.05
Female	0	2.7±0.1	0.23±0.00	0.64±0.05	0.40±0.05	0.72±0.08
	0.01	2.7±0.1	0.21±0.01	0.47±0.01[a]	0.34±0.03	0.65±0.01
	0.05	3.7±0.6	0.23±0.01	0.52±0.00[a]	0.42±0.05	0.94±0.07
	0.25	3.2±0.1	0.21±0.01	0.58±0.02	0.47±0.10	0.95±0.05

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/gm brain weight) Liver	Spleen	Kidneys	Heart
Male	0	6.7±0.3	0.39±0.03	1.50±0.10	0.67±0.03
	0.01	5.3±0.7	0.35±0.06	1.10±0.14	0.74±0.09
	0.05	6.1±0.6	0.47±0.10	1.13±0.11	0.69±0.06
	0.25	6.4±0.2	0.37±0.04	1.33±0.06	0.71±0.04
Female	0	3.8±0.4	0.33±0.04	0.91±0.08	0.56±0.04
	0.01	4.1±0.1	0.32±0.01	0.72±0.01	0.52±0.04
	0.05	3.9±0.5	0.26±0.03	0.56±0.05[a]	0.45±0.04
	0.25	3.4±0.2	0.23±0.03	0.62±0.03[a]	0.49±0.09

Entries are mean ± standard error of four rats.

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4]).

TABLE 47

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED 2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER 4 WEEKS

Sex	% 2,6-DNT in Feed	Body Weight (gm)	Absolute Weights (gm) Liver	Spleen	Kidneys	Heart	Brain
Male	0	530±21	14.7±1.0	0.74±0.04	3.59±0.14	1.73±0.18	2.03±0.07
	0.01	502±10	16.3±0.8	0.89±0.08	3.25±0.21	1.46±0.04	2.16±0.04
	0.05	425±4[a]	15.4±0.3	0.83±0.10	3.10±0.09	1.37±0.08	2.12±0.09
	0.25	309±7[a]	15.2±0.6	0.73±0.04	3.10±0.09	1.22±0.09[a]	2.13±0.10
Female	0	302±7	8.2±0.7	0.54±0.03	2.01±0.12	1.08±0.06	2.08±0.04
	0.01	380±9	8.4±0.4	0.59±0.05	1.76±0.07	1.01±0.13	2.01±0.06
	0.05	250±9[a]	8.3±0.3	0.59±0.06	1.59±0.07[a]	0.89±0.03	2.02±0.03
	0.25	178±6[a]	7.5±0.4	0.41±0.03	1.61±0.08[a]	0.82±0.04	1.93±0.06

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/100 gm body weight) Liver	Spleen	Kidneys	Heart	Brain
Male	0	2.8±0.1	0.14±0.00	0.68±0.01	0.33±0.04	0.38±0.02
	0.01	3.2±0.1[a]	0.18±0.02	0.65±0.03	0.29±0.01	0.43±0.01
	0.05	3.6±0.1[a]	0.20±0.02	0.73±0.02	0.32±0.02	0.50±0.02[a]
	0.25	4.9±0.1[a]	0.24±0.01[a]	1.01±0.02[a]	0.40±0.03	0.69±0.02[a]
Female	0	2.7±0.2	0.18±0.01	0.67±0.04	0.36±0.02	0.69±0.03
	0.01	3.0±0.1	0.21±0.01	0.63±0.02	0.36±0.04	0.72±0.02
	0.05	3.3±0.1[a]	0.24±0.03	0.64±0.02	0.36±0.02	0.81±0.03
	0.25	4.2±0.1[a]	0.23±0.01	0.90±0.04[a]	0.46±0.01	1.09±0.06[a]

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/gm brain weight) Liver	Spleen	Kidneys	Heart
Male	0	7.3±0.7	0.37±0.03	1.78±0.10	0.86±0.11
	0.01	7.5±0.4	0.41±0.03	1.51±0.11	0.68±0.03
	0.05	7.3±0.4	0.40±0.06	1.47±0.09	0.65±0.05
	0.25	7.2±0.2	0.34±0.01	1.46±0.04	0.57±0.03[a]
Female	0	4.0±0.3	0.26±0.01	0.97±0.06	0.52±0.02
	0.01	4.2±0.2	0.28±0.02	0.88±0.04	0.51±0.08
	0.05	4.1±0.1	0.29±0.03	0.79±0.04[a]	0.44±0.02
	0.25	3.9±0.3	0.21±0.02	0.84±0.03	0.43±0.03

Entries are mean ± standard error of four rats.

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4]).

TABLE 48

SUMMARY OF TISSUE LESIONS IN MALE RATS
FED 2,6-DNT FOR 4 WEEKS

Lesions[a]	Dose (% in Feed)															
	0				0.01				0.05				0.25			
Rat No.:	113	114	115	116	138	139	140	141	163	164	165	166	188	189	190	191
Heart																
Focal myocarditis					1											
Lung																
Chronic murine pneumonia									1		1					1
Liver																
Focal mononuclear infiltration				1	1	1	1	1	1	1	1	±	1	1	±	
Extramedullary hematopoiesis													1	1	1	1
Bile duct hyperplasia																1
Cecum																
Pinworms								+								
Testis																
Atrophy																
Degeneration and retardation of spermatogenesis							1			1		1	2	3	2	3
Brain																
Focal hemorrhage			1													
Spleen																
Extramedullary hematopoiesis									1	±	±	±	2	3	3	2
Bone Marrow																
M/E Ratio	1.3	1.3	1.5	1.4	-	1.3	1.0	1.1	1.1	-	1.3	1.4	1.3	1.3	1.3	1.2

Tissues not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

TABLE 49

SUMMARY OF TISSUE LESIONS IN FEMALE RATS
FED 2,6-DNT FOR 4 WEEKS

Lesions[a/]	Rat No.:	Dose (% in Feed) 0				0.01				0.05				0.25			
		213	214	215	216	238	239	240	241	263	264	265	266	288	289	290	291
Heart																	
Focal myocarditis																	1
Lung																	
Chronic murine pneumonia		1			1		±		1	2						1	1
Chronic perivasculitis				2	1					2						1	
Liver																	
Focal mononuclear infiltration						±	1	±	1	1	±	1	1	1			1
Extramedullary hematopoiesis														±	±	±	1
Kidney																	
Microcalculi in medulla						1											
Spleen																	
Extramedullary hematopoiesis						1	1	±	1	±	1	±	±	1	1	1	2
Bone Marrow																	
M/E Ratio		1.6	1.3	1.5	1.3	1.1	1.5	1.1	1.1	1.3	1.3	1.8	1.7	1.3	1.4	1.3	1.4

Tissues not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

TABLE 50

SUMMARY OF TISSUE LESIONS IN MALE RATS
FED 2,6-DNT FOR 13 WEEKS

Lesions[a/]	Dose (% in Feed)											
	0				0.01				0.05			
Rat No.:	105	106	107	108	130	131	132	133	155	156	157	158
Heart												
Focal myocarditis		1										
Trachea												
Tracheitis		3										
Lung												
Chronic murine pneumonia		1										
Submaxillary Salivary Gland												
Sialoadenitis							3					
Liver												
Focal mononuclear infiltration	1	1			1	1				1		
Extramedullary hematopoiesis									±	±	1	1
Bile duct hyperplasia									±		1	±
Focal degeneration												
Kidney												
Dilatation of pelvis and/or tubules												
Testis												
Focal atrophy				2				2				
Degeneration and retardation of spermatogenesis												1
Atrophy and aspermatogenesis												
Spleen												
Hemosiderosis					1	1	1	±				
Extramedullary hematopoiesis									1	±	±	1
Bone Marrow												
M/E Ratio	1.4	1.5	-	1.4	-	-	-	-	-	-	-	-

Lesions[a/]	Dose (% in Feed)			
	0.25			
Rat No.:	180	181	182	183
Heart				
Focal myocarditis				
Trachea				
Tracheitis				
Lung				
Chronic murine pneumonia				
Submaxillary Salivary Gland				
Sialoadenitis				
Liver				
Focal mononuclear infiltration			1	
Extramedullary hematopoiesis	1	1	1	1
Bile duct hyperplasia	1	1	1	1
Focal degeneration		1		
Kidney				
Dilatation of pelvis and/or tubules		1		1
Testis				
Focal atrophy				
Degeneration and retardation of spermatogenesis				
Atrophy and aspermatogenesis	4	4	4	4
Spleen				
Hemosiderosis	3	3	3	2
Extramedullary hematopoiesis	1	1	1	±
Bone Marrow				
M/E Ratio	1.4	1.3	1.5	1.3

Tissues not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

TABLE 51

SUMMARY OF TISSUE LESIONS IN FEMALE RATS
FED 2,6-DNT FOR 13 WEEKS

Lesions[a/]	Dose (% in Feed)											
	0				0.01				0.05				0.25			
Rat No.:	205	206	207	208	230	231	232	233	255	256	257	258	280	281	282	283
Liver																
Focal mononuclear infiltration		±	1		1		±				1	1			1	
Extramedullary hematopoiesis										1		±	1	1	1	±
Bile duct hyperplasia									±	±	±		1	1	1	1
Focal degeneration													1			
Kidney																
Microcalculi		±												1		
Focal mononuclear infiltration			1													1
Uterus																
Infiltration of eosinophils			1	1												
Spleen																
Hemosiderosis			1	2	1	±	2	2					3	3	3	3
Extramedullary hematopoiesis									±	1	1	±	1	1	1	1
Bone Marrow																
M/E Ratio	1.5	-	1.6	1.4	-	-	-	-	-	-	-	-	-	1.5	-	-

Tissues not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

TABLE 52

SUMMARY OF TISSUE LESIONS OF MALE RATS FED
2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a/]	Dose (% in Feed)															
	0				0.01				0.05				0.25			
Rat No.:	109	110	111	112	134	135	136	137	159	160	161	162	184	185	186	187
Heart																
Focal mononuclear infiltration													1			
Lung																
Focal mononuclear infiltration	±		2													1
Focal perivasculitis															1	
Stomach																
Eosinophilic infiltration															1	
Liver																
Focal mononuclear infiltration	1	1		1	1		1	1		1			1	1		
Extramedullary hematopoiesis															±	
Kidney																
Dilatation of tubules and/or pelvis		2	1	2									1	1	1	1
Focal mononuclear infiltration																1
Testis																
Focal atrophy													3	1		1
General atrophy															4	
Focal regeneration															1	
Bone Marrow																
M/E Ratio	1.8	1.7	1.8	-	-	-	-	-	-	-	-	-	1.5	1.6	1.4	1.2

Tissues not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 - marked; 4 = markedly severe.

TABLE 53

SUMMARY OF TISSUE LESIONS OF FEMALE RATS FED
2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a/]	Dose (% in Feed)															
	0				0.01				0.05				0.25			
Rat No.:	209	210	211	212	234	235	236	237	259	260	261	262	284	285	286	287
Lung																
Focal mononuclear infiltration			1										2	1		
Focal perivasculitis	1	1														
Chronic murine pneumonia			1													
Focal emphysema															1	
Liver																
Focal mononuclear infiltration					1	1	1		1			1				
Focal hemorrhage	1															
Focal fatty change	1														1	
Extramedullary hematopoiesis													1	1		±
Kidney																
Dilatation of tubules and/or pelvis			1										2	1	1	1
Microcalculi	1	±														
Uterus																
Eosinophilic infiltration			3											2		
Bone Marrow																
M/E Ratio	1.6	1.5	1.6	1.5	-	-	-	-	-	-	-	-	-	-	1.6	1.8

Tissues not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

TABLE 54

SUMMARY OF TISSUE LESIONS OF MALE RATS FED
2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a/]	Dose (% in Feed)											
	0				0.01				0.05				0.25			
Rat No.:	101	102	103	104	126	127	128	129	151	152	153	154	176	177	178	179
Heart																
Focal myocarditis													2			
Focal mononuclear infiltration																1
Lung																
Chronic murine pneumonia			1	±												
Liver																
Focal mononuclear infiltration	1		1			1	1			1		1				
Fatty change					1						1	1	1	1	1	2
Focal degeneration									1	±		1	2	2	1	2
Extramedullary hematopoiesis					±				±	±			1	1	1	±
Bile duct hyperplasia													±		1	±
Pancreas																
Hemosiderosis														1		
Cecum																
Pinworms	+															
Kidney																
Focal mononuclear infiltration		±											1			
Dilatation of pelvis and/or tubules													1			
Focal necrosis														2		
Tubular casts														1		
Testis																
Atrophy and aspermatogenesis		3										2	4	4	4	3
Degeneration									±		1					
Regeneration															1	2
Brain																
Focal hemorrhage	1															
Spleen																
Hemosiderosis	1	1	1	1				1					3	3	2	
Extramedullary hematopoiesis					±	1	±		1	1	1	1	1	2	1	1
Bone Marrow																
M/E Ratio	1.4	-	1.3	1.5	-	-	-	-	-	-	-	-	1.6	1.4	1.3	1.5

Tissues not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

TABLE 55

SUMMARY OF TISSUE LESIONS OF FEMALE RATS FED
2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a/]	Dose (% in Feed)															
	0				0.01				0.05				0.25			
Rat No.:	201	202	203	204	226	227	228	229	251	252	253	254	276	277	278	279
Heart																
Focal mononuclear infiltration		1											1			
Lung																
Chronic murine pneumonia	1															
Liver																
Focal mononuclear infiltration			1	1		1	1	1			1	1		1		
Portal inflammation		1														
Fatty change								±			1		1	±	1	
Focal degeneration													1	±	1	1
Extramedullary hematopoiesis											±		1	1	1	1
Bile duct hyperplasia													±	1	±	±
Kidney																
Dilatation of tubules and/or pelvis	1			1												
Microcalculi	1			2												
Spleen																
Hemosiderosis					1	3	1	1	2				2	3	3	3
Extramedullary hematopoiesis						±					1	1	±	1	1	1
Bone Marrow																
M/E ratio	1.5	1.5	1.2	1.4	-	-	-	-	-	-	-	-	1.3	1.3	1.6	1.4

Tissues not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

TABLE 56

NUMERICAL DISTRIBUTION OF CHROMOSOMES FROM RATS FED 0.05% OF 2,6-DNT IN THE DIET

Treatment	No. of Rats	Chromosome Frequency ≤40	41	42	43	≥44	Tetraploids/per 100 Cells
Control							
Lymphocytes	4	3[a]	5	40	2	0	0.26 ± 0.15[b]
Kidneys	4	4	5	39	2	0	0 48 ± 0.18
2,6-DNT for 6 weeks							
Lymphocytes	4	2	7	39	2	0	0.75 ± 0.32
Kidneys	2	5	4	40	1	0	1.15 ± 0.55
2,6-DNT for 13 weeks							
Lymphocytes	2	3	6	40	1	0	0.0 ± 0.0
Kidneys	4	4	5	38	2	1	1.25 ± 0.25[c]

a/ Mean

b/ Mean ± S.E.

c/ Significantly different from control, $p < 0.05$ (Student's t test).

TABLE 57

MORPHOLOGICAL ABERRATIONS OF CHROMOSOMES FROM RATS FED 0.05% OF 2,6-DNT IN THE DIET

Treatment	No. of Rats	Chromatid Breaks and Gaps per 50 Cells	Translocations per 50 Cells	Total Aberrations per 50 Cells
Control				
Lymphocytes	4	0.75 ± 0.25[a]	0.25 ± 0.25	1.00 ± 0.40
Kidneys	4	1.50 ± 0.29	0.50 ± 0.29	2.00 ± 0.41
2,6-DNT for 6 weeks				
Lymphocytes	4	2.42 ± 0.57[b]	0.0 ± 0.0	2.42 ± 0.57[b]
Kidneys	2[d]	2.00 ± 1.00	0.0 ± 0.0	2.00 ± 0.00
2,6-DNT for 13 weeks				
Lymphocytes	2[d]	2.00 ± 0.0[c]	0.50 ± 0.50	2.50 ± 0.50[c]
Kidneys	4	2.75 ± 0.48	0.50 ± 0.50	3.25 ± 0.75

a/ Mean ± S.E.
b/ Significantly different from control, $p < 0.01$ (Chi Square test).
c/ Significantly different from control, $p < 0.05$ (Chi Square test).
d/ Two cultures failed to produce sufficient cells in metaphase.

TABLE 58

SERUM IgE (IU/ml) OF RATS FED 0.25% OF 2,6-DNT

Treatment	Weeks of Treatment			
	4	8[b]	13	17[c]
Control	1369±214(4)[a]	--	1206±114(4)	1075±43(4)
2,6-DNT	1165±110(8)	563±82(8)	1034±106(8)	997±75(8)

a/ Mean ± standard error (number of rats).
b/ 2,6-DNT feeding for 4 weeks, then recovery for 4 weeks.
c/ 2,6-DNT feeding for 13 weeks, then recovery for 4 weeks.

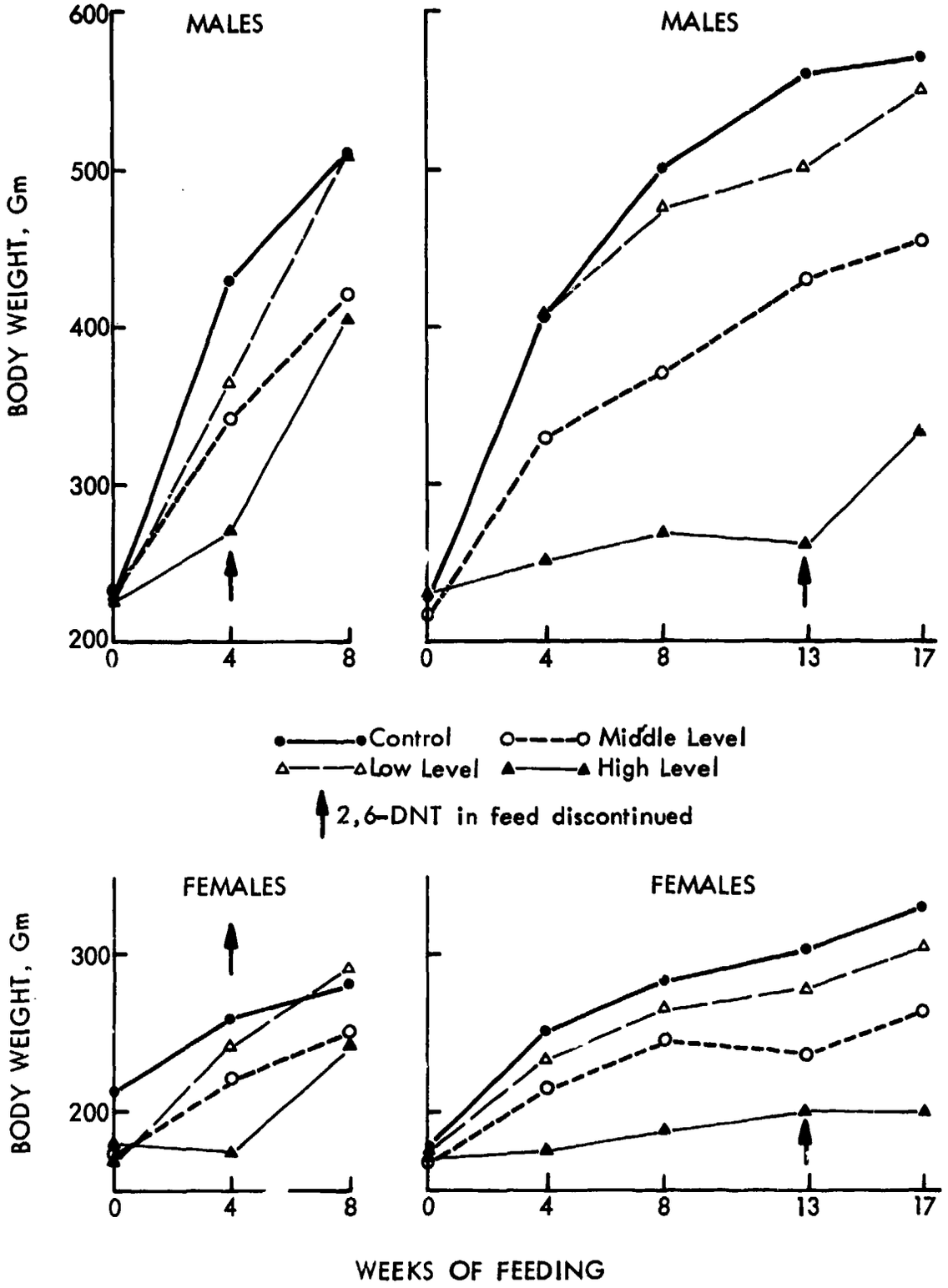

Figure 1 - Body Weights of Rats Fed Various Levels of 2,6-DNT

III. MICE

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III. MICE

A. Subacute and Subchronic Toxicities and Reversibility

1. Introduction

As for the dogs and rats, these studies were performed to define the nature and extent of effects of 2,6-DNT on the biological system at the biochemical and cellular levels and to elucidate the dose-response relationship in mice fed 2,6-DNT for 4 and 13 weeks. The reversibility of any adverse effects was also studied in mice after the feeding of 2,6-DNT was discontinued for 4 weeks.

2. Material and Methods

The basic design and procedure for these experiments in mice were similar to those described for rats in Section II.A.2. with the following exceptions:

a. A total of 64 male and 64 female young healthy albino Swiss mice (National Laboratory Animals, O'Fallon, Missouri) were used for this study. They were divided into four groups, each consisting of 16 males and 16 females. The average weights of all groups were kept close. Three groups of mice were fed 0.01, 0.05 or 0.25% of 2,6-DNT in powdered standard rodent chow (Wayne Laboratory Meal). The fourth group served as controls and was given the powdered standard rodent chow.

b. Mice were kept in a separate room of our rodent quarters. They were housed four per plastic cage with filter tops.

c. Blood samples were collected by heart puncture under ether anesthesia at termination for hematology. Clinical blood chemistry tests in mice were not performed.

3. Results

a. General Observations and Weight Gain

Although no unusual symptoms were observed, there were a number of unscheduled deaths. Three male mice in the control group died during week 12 of no apparent cause. Two males fed the low dosage level (0.01%) were found dead in weeks 1 and 3, respectively, with traumatic lesions from fighting. Unscheduled deaths in the middle dosage group (0.05%) included two males in week 1, four males and one female in week 3, one male in week 10, and one male in week 14, just after being placed on control feed for the recovery study. Of the mice fed the high level

(0.25%), one female died in week 2, one male and two females in week 6, three males and one female in week 7, two males in week 8, and the last two surviving males and two females in week 9. Most of these mice from the middle and high dosage groups had lost weight in the weeks prior to their deaths. To summarize, the total unscheduled deaths include three males in the control group, two males in the low dosage group, eight males and one female in the middle dosage group, and eight males and six females in the high dosage group. Most of these deaths occurred at night. When the bodies were found, autolysis precluded obtaining organ weights and made pathological examination very difficult.

Body weights of male and female mice before, during and after feeding of 2,6-DNT are summarized in Tables 59 and 60, respectively. These data are shown graphically in Figure 2. All mice fed the high level and some mice fed the middle level had decreased weight gain or weight loss. Many mice showing weight losses died. During the recovery period, the surviving high dosage mice regained the lost weight.

b. Feed Consumption and 2,6-DNT Intake

The feed consumption of the mice fed 2,6-DNT is summarized in Table 61. Some feed was scattered about the cage, particularly during the first weeks of the study. Thus, the true feed consumption was less than the apparent amounts. Mice fed the high level of 2,6-DNT (0.25%) usually consumed less feed than the others. During the recovery periods, the females fed the high level ate more than the other groups.

The 2,6-DNT intake of these mice is summarized in Table 62. The 2,6-DNT intake of the male mice fed the low (0.01%), middle (0.05%) and high (0.25%) levels averaged 11.1, 50.8 or 288.8 mg/kg/day, respectively; and that of the female mice averaged 11.0, 55.2 or 298.5 mg/kg/day, respectively.

c. Blood Analysis

The hematology results from the male control mice and those fed various levels of 2,6-DNT are summarized in Tables 63 through 66, and the female controls and those fed 2,6-DNT are summarized in Tables 67 through 70. Some blood samples clotted, making it impossible to perform some or all of the tests; standard errors were not calculated on fewer than three samples. There were a number of statistically significant changes when the various parameters were compared with the controls at the respective time intervals. However, the changes were mild, inconsistent and not related to 2,6-DNT.

d. Organ Weights

The organ weights of the mice fed various levels of 2,6-DNT are summarized in Tables 71 through 74. There were a number of statistically significant variations in absolute and relative organ weights when compared with those of the control mice. However, there were no consistent changes between sexes, doses or treatment periods, reflecting the natural variations inherent in the animals. Mice dying at unscheduled times usually had low body weights, but autolysis precluded reliable determination of the organ weights.

e. Gross and Microscopic Examination of Tissues

At necropsy, most mice fed the high level of 2,6-DNT (0.25%) and some fed the middle level (0.05%) had only little fat. Some male mice dying at unscheduled times had traumatic lesions due to fighting. Otherwise, the mice appear grossly normal and in good nutritional condition at necropsy.

The results of tissue lesions in male and female control mice and those fed 2,6-DNT are shown in Tables 75 through 82. Only the organs likely to have 2,6-DNT related lesions, including liver, testes, spleen and those with gross lesions from the low and middle dosage mice were examined microscopically. A number of spontaneous lesions were observed in the liver, kidney or other tissues of the male and female control mice and mice fed 2,6-DNT. Several mice had pinworms in the intestine. Nonactive ovaries were seen in a number of females, reflecting immaturity in these mice. Several mice had focal mononuclear infiltration or mild perivascular cuffing in the brain. These lesions were seen in the controls as well as in the treated mice. They were not related to 2,6-DNT. In addition, one male fed the low level for 4 weeks had focal areas of bronchiogenic carcinoma in one lobe of the lung. The tumor appeared mildly invasive. Other occasional lesions seen in one or more mice fed 2,6-DNT included focal epicarditis or myocarditis, focal cystitis, chronic murine pneumonia or bronchopneumonia, metritis and focal myositis. These lesions were incidental and were not caused by 2,6-DNT. The bone marrows of these mice were normal and their M/E ratios of the rib marrows were within normal ranges.

Three types of lesions seen in these mice were related to 2,6-DNT. Marked aspermatogenesis was seen in all males fed the high level of 2,6-DNT for 4 weeks (Table 75). No males fed this level survived more than 8 weeks. Depression of spermatogenesis was also seen in one male fed the middle level for 4 weeks and in two males fed the low level for 4 or 13 weeks (Tables 75 and 77). Bile duct hyperplasia was developed in the only surviving mouse fed the high level of 2,6-DNT for 13 weeks and in

two mice fed the middle level for 13 weeks (Tables 77 and 78). This lesion was also seen in two mice fed the high level for 4 weeks and allowed to recover for 4 weeks (Tables 79 and 80). This probably suggests the slow development of this lesion. Extramedullary hematopoiesis in the liver and spleen was seen more often in mice fed 2,6-DNT than in the controls. In general, the incidence and probably the severity of this lesion were in proportion to the level of 2,6-DNT in the feed.

When the mice were allowed to recover for 4 weeks after being treated for 4 weeks, the testicular lesions were not seen in any high dosage males (Table 79). As discussed above, the high dosage males did not survive for more than 8 weeks. Whether or not the testicular lesions will be reversible after treatment for longer periods is not known. Also as discussed above, bile duct hyperplasia was seen in some mice allowed to recover for 4 weeks (Tables 79 and 80). In addition, extramedullary hematopoiesis in the liver or spleen continued to occur in treated mice after recovery for 4 weeks.

4. Discussion and Conclusions

The 2,6-DNT intake of the male mice fed the low, middle or high level of 2,6-DNT in the feed averaged 11.1, 50.8 or 288.8 mg/kg/day, respectively; and that of the female mice averaged 11.0, 55.2 or 298.8 mg/kg/day, respectively.

There were two deaths among the control mice and three deaths among the low dosage level mice. In the middle and high dosage groups, there were nine and 14 deaths, respectively; most, if not all, of these deaths were due to 2,6-DNT. The males were somewhat more susceptible, accounting for eight deaths among each of these two dosage levels. Furthermore, all males fed the high level died before week 9, while two of eight females survived the full 13 weeks of feeding. Neither characteristic symptoms nor other definite signs of imminent death were observed. The dead mice were usually of low body weight, frequently with significant weight losses a week or two before death. However, some mice lost weight and then recovered somewhat while continuing to take the 2,6-DNT.

No toxicologically significant changes were seen in the peripheral blood elements. Contributing to this negative result in part were the clotted samples. Furthermore, the most severely affected mice died overnight and no blood samples could be taken.

The histopathological lesions seen were similar to those discussed in dogs and rats. There were testicular atrophy with depression of spermatogenesis, bile duct hyperplasia and extramedullary hematopoiesis in the liver and/or spleen. The hematopoietic effect presumably reflects a

response to a toxic anemia which was not directly seen. These lesions were generally mild as compared to the other species. The mice nearly recovered in 4 weeks when 2,6-DNT in feed was discontinued. Two mice fed 0.25% of 2,6-DNT for 4 weeks and allowed to recover 4 weeks had bile duct hyperplasia, whereas none of those necropsied after feeding for 4 weeks had such a lesion. If the lesion is slow to develop and slow to regress, the observation may suggest the late appearance of this lesion.

In the 2,4-DNT studies,[6] the mice were almost unaffected by a dose that was lethal to rats. However, in this study mice were more susceptible than rats to 2,6-DNT. Similar affects were observed in LD_{50} studies.[1] One possible explanation is that the mouse absorbs 2,6-DNT well. We have shown that the mouse absorbed 2,4-DNT poorly, while the rat, rabbit, dog and rhesus monkey absorbed it well.[6]

B. Other Studies

Mutagenic and immunologic studies were not performed in mice.

C. Summary

The 2,6-DNT intake of the males fed the low, middle or high level of 2,6-DNT in the feed averaged 11, 51 and 289 mg/kg/day, respectively. The females consumed an average of 11, 55 and 299 mg/kg/day, respectively. The low level produced no effects. The middle or the high level produced decreases in feed consumption and weight gain, extramedullary hematopoiesis, depression of spermatogenesis and atrophy in the testes, bile duct hyperplasia and death. Mice fed the high level were more severely affected than those fed the middle level. As for dogs and rats, there was partial recovery in mice 4 weeks after cessation of treatment.

TABLE 59

BODY WEIGHTS OF MALE MICE FED 2,6-DNT

% 2,6-DNT in Feed	Body Weights (gm) [a]				
	Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	24.8±0.5	26.5±0.5			
0.01	24.0±1.4	25.0±0.5			
0.05	22.3±0.9	27.3±1.2			
0.25	23.5±1.6	22.5±2.3			
0	23.5±1.0	25.8±1.3 [b]	29.8±1.0		
0.01	25.8±1.9	25.8±2.3 [b]	32.5±1.5		
0.05	24.0±0.6	[c]	[c]		
0.25	20.5±2.1	21.5±2.8 [b]	29.3±1.8		
0	23.5±0.7	23.8±1.0	26.5±1.7	32.5 [d]	
0.01	26.3±1.0	27.5±0.7	33.0±1.3	36.7±1.6	
0.05	24.0±2.8	27.0±2.0 [e]	34.0±1.2 [e]	31.3±2.2 [e]	
0.25	26.0±1.5	23.3±1.7	24 [f]	[g]	
0	24.8±0.5	21.5±1.0	31.0±1.2	29.3±0.7 [b,h]	34.0±1.2 [h]
0.01	24.0±1.4	28.5 [i]	30.5 [i]	31.0 [b,i]	33.0 [i]
0.05	21.8±1.3	27.3±2.7 [e]	30.3±4.3 [e]	28.0 [b,j]	32.0 [k]
0.25	25.0±0.4	23.0±1.8	22 [l]	[g]	[g]

[a] Mean ± S.E. of 4 unless otherwise noted.
[b] 2,6-DNT in feed discontinued thereafter.
[c] All mice died in week 3.
[d] Two mice; two other mice died in week 12.
[e] Three mice; one other mouse died in week 1.
[f] One mouse; one other mouse each died in week 6, 7 and 8.
[g] Last mouse died week 9.
[h] Three mice; one other mouse died in week 12.
[i] Two mice; one other mouse each died in week 1 and 3.
[j] Two mice; one other mouse died in week 10.
[k] One mouse; one other mouse died in week 14.
[l] One mouse; two other mice died in week 7 and one in week 8.

TABLE 60

BODY WEIGHTS OF FEMALE MICE FED 2,6-DNT

% 2,6-DNT in Feed	Body Weights (gm) [a]				
	Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	19.6±1.3	21.3±1.1			
0.01	21.5±1.0	22.3±0.9			
0.05	19.8±0.3	23.8±0.9			
0.25	21.8±1.3	20.8±3.0			
0	20.0±0.8	21.3±1.0 [b]	26.3±2.0		
0.01	21.8±1.0	24.0±1.1 [b]	26.3±1.7		
0.05	21.5±0.7	21.3±0.5 [b]	23.0±1.1		
0.25	21.5±0.9	20.8±0.3 [b]	26.5±1.7		
0	21.3±1.0	24.3±1.0	27.8±1.4	29.5±2.5	
0.01	20.3±1.3	21.0±1.2	23.5±1.2	24.5±2.3	
0.05	21.3±1.0	26.8±1.0	26.8±1.0	27.8±1.0	
0.25	19.5±0.9	18.3±0.3 [c]	20.0 [d]	25.0 [d]	
0	24.3±1.5	22.0±0.8	26.8±1.7	29.8±0.3 [b]	31.8±1.8
0.01	18.5±0.9	23.0±0.7	26.5±1.3	26.5±1.9 [b]	28.5±1.7
0.05	19.8±0.3	25.0±1.0 [c]	28.3±0.3 [c]	31.3±0.7 [b,c]	31.3±1.2 [c]
0.25	20.0±1.5	25.5±1.0	23.3±2.2 [e]	28.0 [b,f]	32.0 [f]

[a] Mean ± S,E, of 4 unless otherwise noted.
[b] 2,6-DNT in feed discontinued thereafter.
[c] Three mice; one other mouse died in week 2.
[d] One mouse; two other mice died in week 6.
[e] Three mice; one other mouse died in week 7.
[f] One mouse; two other mice died in week 9.

TABLE 61

AVERAGE FEED CONSUMPTION (gm/mouse/day) OF MICE FED 2,6-DNT

% 2,6-DNT in Feed	Males				
	1-4 [a]	5-8	5-8 [b]	9-13	14-17 [b]
0	3.50	2.53	2.41	2.41	2.54
0.01	3.65	2.77	2.46	3.08	3.37
0.05	3.74	2.48	----	2.39	1.77
0.25	3.02	2.42	2.29	----	----
	Females				
	1-4	5-8	5-8 [b]	9-13	14-17 [b]
0	3.29	2.37	2.11	2.43	2.83
0.01	3.48	1.99	1.98	2.26	2.28
0.05	3.23	2.83	2.22	2.34	2.37
0.25	2.56	1.92	2.56	3.45	4.01

a/ In weeks.

b/ Recovery period after 2,6-DNT in feed discontinued.

TABLE 62

AVERAGE 2,6-DNT INTAKE (mg/kg/day) OF MICE DURING TREATMENT

% 2,6-DNT in Feed	Males			
	1-4 [a]	5-8	9-13	1-13
0.01	14.1	9.2	9.5	11.1
0.05	75.5	41.5	37.0	50.8
0.25	326.3	262.0	-----	288.8
	Females			
	1-4	5-8	9-13	1-13
0.01	15.8	8.5	8.9	11.0
0.05	72.0	53.0	40.5	55.2
0.25	306.8	232.5	350.0	298.5

a/ Weeks.

TABLE 63

LABORATORY DATA OF MALE MICE FED 2,6-DNT FOR 4 WEEKS

(C,N) CONTROL
(T,N) TREATMENT
N = NUMBER OF MICE

DOSE (% 2,6-DNT IN FEED):	0 (C, 4)	0.01 (T, 4)	0.05 (T, 4)	1 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.76 ± .18	5.82 ± .29	6.20 ± .14	6.94 ± .37[a/]
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.32 ± .56
RETICULOCYTES, %	2.11 ± .40	3.23 ± .74	2.40 ± .10	2.22 ± .25
HEMATOCRIT, VOL. %	39.3 ± 2.6	38.3 ± 2.2	41.0 ± 1.5	42.0 ± 1.5
HEMOGLOBIN, GM. %	13.4 ± .5	12.6 ± .4	13.9 ± .3	14.1 ± .5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.1 ± 1.2
MCV, CUBIC MICRONS	68.2 ± 2.6	66.4 ± 6.5	66.3 ± 3.2	60.8 ± 2.4
MCHH, MICRO MICROGMS.	23.3 ± .3	21.7 ± 1.8	22.5 ± .7	20.5 ± .6
MCHHC, GM %	34.2 ± 1.0	32.9 ± 1.5	34.1 ± .7	33.7 ± .5
PLATELETS ($X10^5$ /MM^3)	5.6	8.7 ± .7	10.2 ± .9[a/]	7.4 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	10.0 ± 2.8	10.8 ± 2.1	7.7 ± 1.2	10.1 ± 1.2
NEUTROPHILS, %	5.3 ± 1.3	19.3 ± 1.3[a/]	21.5 ± 2.1[a/]	24.5 ± 3.2[a/]
LYMPHOCYTES, %	94.5 ± 1.2	77.0 ± 1.6[a/]	76.3 ± 2.3[a/]	72.0 ± 4.0[a/]
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	.5 ± .5	0.0 ± 0.0	0.0 ± 0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	3.3 ± .5[a/]	2.3 ± .3	3.5 ± .9[a/]
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the control mice (Dunnett's multiple comparison procedure[4/]).

TABLE 64

LABORATORY DATA OF MALE MICE FED 2,6-DNT FOR 13 WEEKS

(C,N) CONTROL
(T,N) TREATMENT
N = NUMBER OF MICE

DOSE (% 2,6-DNT IN FEED):	0 (C, 1)	0.01 (T, 3)	0.05 (T, 3)
ERYTHROCYTES ($X10^6$ /MM^3)	6.31	6.99 ± .01	7.05 ± .25
HEINZ BODIES, %	0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	2.74	1.72 ± .09	1.87 ± .35
HEMATOCRIT, VOL, %	40.0	46.7 ± 1.2	44.3 ± .3
HEMOGLOBIN, GM, %	12.9	15.7 ± .2	15.1 ± .2
METHEMOGLOBIN, %	0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	63.4	66.3 ± 1.7	63.0 ± 2.3
MCHH, MICRO MICROGMS,	20.4	22.5 ± .2	21.4 ± .6
MCHHC, GM %	32.2	34.0 ± .5	34.1 ± .7
PLATELETS ($X10^5$ /MM^3)	8.9	8.7 ± .7	7.3 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	9.6	13.1 ± 2.2	13.1 ± 1.4
NEUTROPHILS, %	28.0	23.7 ± 1.3	25.7 ± 8.2
LYMPHOCYTES, %	70.0	74.3 ± 2.3	73.3 ± 8.7
BANDS, %	0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0	0.0 ± 0.0	0.0 ± 0.0
BASOPHILS, %	0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	2.0	2.0 ± 1.5	1.0 ± .6
ATYPICAL, %	0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR OR ONLY VALUE

TABLE 65

LABORATORY DATA OF MALE MICE FED 2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C,N) CONTROL
(T,N) TREATMENT
N = NUMBER OF MICE

DOSE (% 2,6-DNT IN FEED):	0 (C, 2)	0.01 (T, 4)	0.25 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	4.23	3.91 ± .57	3.72 ± .30
HEINZ BODIES, %	0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.80	1.82 ± .34	2.26 ± .43
HEMATOCRIT, VOL, %	40.5	40.0 ± .7	37.5 ± 2.2
HEMOGLOBIN, GM, %	13.9	10.6 ± 2.2	12.7 ± .5
METHEMOGLOBIN, %	0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	96.1	107.0 ± 11.1	101.6 ± 4.9
MCHH, MICRO MICROGMS,	33.0	30.5 ± 7.9	34.4 ± 1.7
MCHHC, GM %	34.5	26.8 ± 5.8	33.9 ± .8
PLATELETS ($X10^5$ /MM^3)	7.4	6.4 ± .9	4.9 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	16.0	17.7 ± 1.1	13.6 ± 1.9
NEUTROPHILS, %	21.0	15.8 ± 3.7	9.8 ± 2.8
LYMPHOCYTES, %	76.5	84.3 ± 3.7	89.5 ± 2.5
BANDS, %	.5	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.0	0.0 ± 0.0	.8 ± .5
BASOPHILS, %	0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR OR MEAN

TABLE 66

LABORATORY DATA OF MALE MICE FED 2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C,N) CONTROL
(T,N) TREATMENT
N = NUMBER OF MICE

DOSE (% 2,6-DNT IN FEED):	0 (C, 3)	0.01 (T, 2)
ERYTHROCYTES ($X10^6$ /MM^3)	7.19	8.02
HEINZ BODIES, %	0.00 ± 0.00	0.00
RETICULOCYTES, %	2.17 ± .53	1.92
HEMATOCRIT, VOL. %	44.5	48.0
HEMOGLOBIN, GM. %	15.1	16.5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0
MCV, CUBIC MICRONS	61.9	59.8
MCHC, MICRO MICROGMS.	21.2	20.6
MCHBC, GM %	34.3	34.4
PLATELETS ($X10^5$ /MM^3)	8.9	9.6
LEUKOCYTES ($X10^3$ /MM^3)	12.2	15.0
NEUTROPHILS, %	32.7 ± 16.2	37.5
LYMPHOCYTES, %	66.0 ± 15.6	60.5
BANDS, %	0.0 ± 0.0	0.0
EOSINOPHILS, %	.3 ± .3	1.0
BASOPHILS, %	0.0 ± 0.0	0.0
MONOCYTES, %	1.0 ± .6	1.0
ATYPICAL, %	0.0 ± 0.0	0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0

ENTRIES ARE MEAN ± STANDARD ERROR OR MEAN

TABLE 67

LABORATORY DATA OF FEMALE MICE FED 2,6-DNT FOR 4 WEEKS

(C,N) CONTROL
(T,N) TREATMENT
N = NUMBER OF MICE

DOSE (% IN FEED):	0 (C, 4)	0.01 (T, 4)	0.05 (T, 4)	0.25 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.14 ± .21	6.71	5.91 ± .51	6.01 ± .32
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	.31 ± .22
RETICULOCYTES, %	2.40 ± .48	1.01 ± .12	4.00 ± 1.22	1.77 ± .59
HEMATOCRIT, VOL, %	38.3 ± .9	45.0	41.0 ± .6	37.8 ± .6
HEMOGLOBIN, GM, %	12.6 ± .3	15.1	13.3 ± .3	12.9 ± .3
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	74.7 ± 3.2	67.5	70.3 ± 5.6	63.2 ± 2.4
MCHB, MICRO MICROGMS.	24.6 ± 1.1	22.6	22.7 ± 1.5	21.6 ± .9
MCHBC, GM %	32.9 ± .2	33.5	32.3 ± .4	34.2 ± .4
PLATELETS ($X10^5$ /MM^3)	4.6 ± .7	6.1 ± .9	5.0 ± .5	6.0 ± 1.0
LEUKOCYTES ($X10^3$ /MM^3)	10.2 ± 1.1	9.8	12.0 ± .9	9.6 ± 2.5
NEUTROPHILS, %	13.8 ± 1.4	18.5 ± 3.4	8.8 ± 2.7	21.0 ± 7.7
LYMPHOCYTES, %	84.5 ± 2.2	80.0 ± 3.1	90.3 ± 2.9	76.8 ± 7.6
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.3 ± .6	.5 ± .3	.8 ± .3	.8 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .3	1.0 ± .6	.3 ± .3	1.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN + STANDARD ERROR OR MEAN

TABLE 68

LABORATORY DATA OF FEMALE MICE FED 2,6-DNT FOR 13 WEEKS

(C,N) CONTROL
(T,N) TREATMENT
N = NUMBER OF MICE

DOSE (% 2,6-DNT IN FEED):	0 (C, 4)	0.01 (T, 4)	0.05 (T, 4)	0.25 (T, 1)
ERYTHROCYTES ($\times 10^6$ /MM^3)	6.66 ± .51	6.93 ± .17	6.95 ± .26	6.76
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
RETICULOCYTES, %	1.53 ± .47	1.29 ± .20	1.75 ± .36	1.48
HEMATOCRIT, VOL. %	43.3 ± 1.8	44.8 ± .5	45.0 ± .7	46.0
HEMOGLOBIN, GM. %	15.0 ± .7	15.7 ± .3	15.6 ± .6	15.5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
MCV, CUBIC MICRONS	65.6 ± 3.0	64.6 ± 1.7	64.9 ± 1.2	68.0
MCHA, MICRO MICROGMS.	22.7 ± .9	22.8 ± .7	22.5 ± .8	22.9
MCHBC, GM %	34.7 ± .3	35.2 ± .2	34.8 ± 1.1	33.7
PLATELETS ($\times 10^5$ /MM^3)	4.7 ± .8	5.8 ± .9	6.8 ± .4	5.4
LEUKOCYTES ($\times 10^3$ /MM^3)	8.3 ± 1.3	9.0 ± .6	11.6 ± .9	10.5
NEUTROPHILS, %	13.8 ± 3.3	12.5 ± 1.5	16.0 ± 7.4	24.0
LYMPHOCYTES, %	80.5 ± 2.4	86.8 ± 1.4	83.5 ± 7.2	76.0
BANDS, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0
EOSINOPHILS, %	1.5 ± 1.0	.5 ± .3	.5 ± .3	0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
MONOCYTES, %	4.0 ± 2.4	.3 ± .3	0.0 ± 0.0	0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0

ENTRIES ARE MEAN ± STANDARD ERROR OR ONLY VALUE

TABLE 69

LABORATORY DATA OF FEMALE MICE FED 2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C,d) CONTROL
(T,N) TREATMENT
N = NUMBER OF MICE

DOSE (% 2,6-DNT IN FEED):	0 (C, 4)	0.01 (T, 4)	0.05 (T, 4)	0.25 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	3.38 ± .10	4.05 ± .25	4.63 ± .39[a/]	4.25 ± .15
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.34 ± .24	1.95 ± .48	1.31 ± .27	2.59 ± .22[a/]
HEMATOCRIT, VOL. %	42.5 ± 1.3	40.8 ± 1.9	43.3 ± 2.2	42.8 ± 1.1
HEMOGLOBIN, GM, %	14.1 ± .6	13.6 ± .6	14.5 ± .6	14.3 ± .4
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	126.2 ± 7.0	100.9 ± 3.0[a/]	94.4 ± 5.0[a/]	106.9 ± 4.4[a/]
MCHH, MICRO MICROGMS,	41.7 ± 2.6	33.6 ± 1.0[a/]	31.7 ± 1.6[a/]	33.8 ± 1.7[a/]
MCHHC, GM %	33.0 ± .4	33.3 ± .3	33.6 ± .5	33.5 ± .3
PLATELETS ($X10^5$ /MM^3)	4.1 ± .9	5.6 ± 1.0	4.3 ± .4	5.9 ± 1.0
LEUKOCYTES ($X10^3$ /MM^3)	12.5 ± 1.7	11.6 ± 1.4	11.0 ± .9	14.9 ± 1.7
NEUTROPHILS, %	12.0 ± 1.8	11.8 ± 3.7	8.0 ± 2.5	8.8 ± 1.9
LYMPHOCYTES, %	88.0 ± 1.8	86.5 ± 3.5	91.8 ± 2.4	90.3 ± 1.7
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	1.3 ± .8	.3 ± .3	.8 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	.5 ± .4	0.0 ± 0.0	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

[a/] Significantly different from the controls (Dunnett's multiple comparison procedure[4/]).

TABLE 70

LABORATORY DATA OF FEMALE MICE FED 2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C,N) CONTROL
(T,N) TREATMENT
N = NUMBER OF MICE

DOSE (% 2,6-DNT IN FEED):	0 (C, 4)	0.01 (T, 4)	0.05 (T, 3)	0.25 (T, 1)
ERYTHROCYTES ($\times 10^6$ /MM^3)	7.56	7.90 ± .30	8.12 ± .37	8.55
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
RETICULOCYTES, %	1.58 ± .21	.71 ± .23[a/]	1.47 ± .14	1.15
HEMATOCRIT, VOL. %	43.0	48.5 ± .6	47.0 ± 1.7	51.0
HEMOGLOBIN, GM. %	15.9	16.5 ± .3	16.2 ± .6	17.0
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	3.1 ± .1	2.9
MCV, CUBIC MICRONS	56.9	61.6 ± 1.8	57.9 ± .8	59.6
MCHH, MICRO MICROGMS.	21.0	21.0 ± .6	20.0 ± .3	19.9
MCHBC, GM %	37.0	34.1 ± .3	34.5 ± .1	33.3
PLATELETS ($\times 10^5$ /MM^3)	8.2	7.2 ± .9	6.1 ± .9	7.2
LEUKOCYTES ($\times 10^3$ /MM^3)	7.1	9.2 ± .3	9.2 ± 1.3	7.6
NEUTROPHILS, %	11.0 ± 4.4	13.3 ± 3.5	13.7 ± .3	8.0
LYMPHOCYTES, %	86.8 ± 5.2	84.5 ± 2.9	85.7 ± .9	92.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
EOSINOPHILS, %	1.5 ± .9	1.0 ± .4	.3 ± .3	0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
MONOCYTES, %	.8 ± .5	1.3 ± .8	.3 ± .3	0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0

ENTRIES ARE MEAN ± STANDARD ERROR, MEAN OR ONLY VALUE

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4/]).

TABLE 71

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED 2,6-DNT FOR 4 WEEKS

Sex	% 2,6-DNT in Feed	Body Weight (gm)	Absolute Weights (gm) Liver	Spleen	Kidneys	Heart	Brain
Male	0	26.0±0.7	1.38±0.06	0.16±0.02	0.52±0.02	0.16±0.01	0.41±0.01
	0.01	26.8±0.8	1.53±0.10	0.18±0.03	0.53±0.03	0.20±0.01	0.48±0.01[a]
	0.05	27.8±1.4	1.46±0.08	0.18±0.02	0.57±0.05	0.19±0.02	0.47±0.02[a]
	0.25	21.5±1.9	1.44±0.07	0.14±0.03	0.42±0.06	0.15±0.01	0.48±0.01[a]
Female	0	23.8±0.9	1.48±0.06	0.31±0.06	0.42±0.02	0.18±0.01	0.46±0.01
	0.01	24.5±1.0	1.40±0.10	0.23±0.04	0.46±0.03	0.20±0.01	0.46±0.02
	0.05	22.3±0.5	1.28±0.04	0.24±0.02	0.40±0.03	0.15±0.02	0.48±0.03
	0.25	19.0±3.0	1.22±0.21	0.16±0.05	0.34±0.06	0.13±0.01	0.43±0.03

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/100 gm body weight) Liver	Spleen	Kidneys	Heart	Brain
Male	0	5.3±0.1	0.63±0.05	2.02±0.09	0.60±0.05	1.59±0.07
	0.01	5.7±0.2	0.66±0.10	2.00±0.11	0.75±0.06	1.78±0.09
	0.05	5.3±0.1	0.66±0.05	2.05±0.12	0.69±0.08	1.71±0.07
	0.25	6.8±0.7[a]	0.61±0.07	1.94±0.15	0.72±0.03	2.26±0.17[a]
Female	0	6.3±0.3	1.28±0.18	1.77±0.05	0.75±0.05	1.95±0.06
	0.01	5.7±0.3	0.93±0.14	1.87±0.08	0.80±0.02	1.86±0.10
	0.05	5.8±0.2	1.08±0.12	1.80±0.08	0.69±0.07	2.14±0.10
	0.25	6.4±0.1	0.77±0.16	1.80±0.12	0.74±0.07	2.42±0.34

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/gm brain weight) Liver	Spleen	Kidneys	Heart
Male	0	3.4±0.2	0.40±0.04	1.28±0.09	0.38±0.03
	0.01	3.2±0.3	0.37±0.06	1.13±0.08	0.42±0.02
	0.05	3.1±0.2	0.39±0.04	1.21±0.09	0.41±0.05
	0.25	3.0±0.2	0.28±0.06	0.89±0.13[a]	0.32±0.02
Female	0	3.2±0.1	0.66±0.11	0.91±0.05	0.39±0.03
	0.01	3.1±0.3	0.50±0.08	1.02±0.09	0.43±0.02
	0.05	2.7±0.2	0.51±0.07	0.84±0.03	0.32±0.04
	0.25	2.8±0.4	0.36±0.09	0.80±0.14	0.31±0.02

Entries are mean ± standard error of four mice.

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4]).

TABLE 72

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED 2,6-DNT FOR 13 WEEKS

Sex	% 2,6-DNT in Feed	Body Weight (gm)	Absolute Weights (gm) Liver	Spleen	Kidneys	Heart	Brain
Male	0	27.5±2.5	1.75±0.46	0.17±0.00	0.87±0.01	0.22±0.03	0.44±0.02
	0.01	32.5±1.6	1.51±0.08	0.15±0.02	1.01±0.10	0.22±0.02	0.43±0.01
	0.05	31.3±2.2	1.54±0.08	0.14±0.01	0.54±0.06	0.22±0.01	0.41±0.03
Female	0	25.0±0.9	1.27±0.07	0.17±0.02	0.41±0.03	0.16±0.01	0.44±0.01
	0.01	22.0±1.4	1.11±0.11	0.12±0.02	0.39±0.03	0.15±0.01	0.41±0.01
	0.05	24.0±1.3	1.40±0.03	0.17±0.03	0.04±0.02	0.16±0.01	0.46±0.01
	0.25	22.0	1.78	0.14	0.39	0.18	0.39

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/100 gm body weight) Liver	Spleen	Kidneys	Heart	Brain
Male	0	6.3±1.1	0.63±0.07	3.19±0.25	0.80±0.17	1.62±0.07
	0.01	4.6±0.1	0.45±0.06	3.11±0.22	0.67±0.04	1.32±0.07[a]
	0.05	4.9±0.3	0.44±0.03	1.73±0.13[a]	0.72±0.05	1.31±0.02[a]
Female	0	5.1±0.3	0.70±0.08	1.66±0.11	0.65±0.01	1.76±0.04
	0.01	5.0±0.2	0.52±0.05	1.75±0.04	0.67±0.04	1.90±0.15
	0.05	5.9±0.2	0.68±0.10	1.69±0.04	0.66±0.02	1.95±0.10
	0.25	8.1	0.65	1.78	0.80	1.75

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/gm brain weight) Liver	Spleen	Kidneys	Heart
Male	0	3.9±9.9	0.38±0.03	1.97±0.07	0.49±0.08
	0.01	3.5±0.2	0.34±0.04	2.36±0.21	0.51±0.04
	0.05	3.8±0.2	0.34±0.03	1.32±0.09	0.55±0.04
Female	0	2.9±0.2	0.40±0.05	0.95±0.08	0.37±0.01
	0.01	2.7±0.3	0.29±0.05	0.95±0.10	0.36±0.03
	0.05	3.0±0.1	0.36±0.07	0.87±0.04	0.34±0.02
	0.25	4.6	0.37	1.02	0.46

Entries are mean ± standard error of two to four mice or single value.

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4]).

TABLE 73

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED 2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Sex	% 2,6-DNT in Feed	Body Weight (gm)	Absolute Weights (gm) Liver	Spleen	Kidneys	Heart	Brain
Male	0	27.5±1.4	1.50±0.01	0.16±0.02	0.56±0.05	0.17±0.01	0.44±0.02
	0.01	33.0±2.3	1.62±0.11	0.18±0.05	0.65±0.05	0.27±0.02[a]	0.44±0.03
	0.25	31.0±1.4	1.34±0.08	0.18±0.01	0.50±0.01	0.20±0.01	0.41±0.02
Female	0	24.3±1.3	1.36±0.06	0.19±0.03	0.46±0.02	0.21±0.02	0.42±0.02
	0.01	26.3±0.9	1.53±0.11	0.28±0.02	0.49±0.01	0.20±0.01	0.45±0.02
	0.05	21.8±0.6	1.34±0.05	0.15±0.02	0.48±0.02	0.17±0.01[a]	0.39±0.02
	0.25	24.3±1.3	1.41±0.06	0.22±0.04	0.44±0.02	0.15±0.01[a]	0.40±0.01

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/gm brain weight) Liver	Spleen	Kidneys	Heart	Brain
Male	0	5.5±0.3	0.59±0.08	2.04±0.12	0.62±0.03	1.63±0.15
	0.01	5.0±0.5	0.54±0.11	1.98±0.03	0.83±0.02[a]	1.35±0.03
	0.25	4.3±0.1	0.58±0.04	1.63±0.09[a]	0.65±0.02	1.32±0.12
Female	0	5.6±0.4	0.76±0.12	1.90±0.12	0.89±0.10	1.73±0.09
	0.01	5.8±0.4	1.09±0.11	1.87±0.06	0.78±0.04	1.73±0.12
	0.05	6.2±0.1	0.68±0.11	2.18±0.03	0.76±0.03	1.79±0.12
	0.25	5.9±0.3	0.89±0.13	1.80±0.08	0.64±0.04[a]	1.67±0.10

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/gm brain weight) Liver	Spleen	Kidneys	Heart
Male	0	3.4±0.1	0.36±0.03	1.28±0.14	0.39±0.03
	0.01	3.7±0.3	0.40±0.08	1.47±0.06	0.62±0.03[a]
	0.25	3.3±0.3	0.44±0.04	1.24±0.04	0.50±0.04
Female	0	3.3±0.1	0.44±0.06	1.09±0.03	0.51±0.04
	0.01	3.4±0.4	0.63±0.03	1.09±0.05	0.45±0.02
	0.05	3.5±0.3	0.38±0.04	1.24±0.07	0.43±0.02
	0.25	3.5±0.2	0.55±0.09	1.09±0.07	0.38±0.02[a]

Entries are mean ± standard error of four mice.

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4]).

TABLE 74

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED 2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Sex	% 2,6-DNT in Feed	Body Weight (gm)	Absolute Weights (gm) Liver	Spleen	Kidneys	Heart	Brain
Male	0	33.7±1.3	1.79±0.07	0.12±0.01	0.67±0.04	0.22±0.03	0.41±0.03
	0.01	31.5±0.5	1.62±0.16	0.19±0.02	0.06±0.04	0.23±0.01	0.43±0.03
	0.05	30.0	1.46	0.11	0.59	0.18	0.39
Female	0	29.8±1.5	1.52±0.10	0.15±0.01	0.49±0.03	0.16±0.01	0.50±0.01
	0.01	26.5±1.7	1.41±0.11	0.13±0.01	0.51±0.02	0.17±0.01	0.49±0.02
	0.05	28.7±0.9	1.55±0.10	0.21±0.01[a/]	0.60±0.02	0.19±0.02	0.53±0.01
	0.25	29.0	1.91	0.16	0.46	0.19	0.45

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/100 gm body weight) Liver	Spleen	Kidneys	Heart	Brain
Male	0	5.3±0.4	0.37±0.02	2.02±0.19	0.64±0.07	1.22±0.04
	0.01	5.1±0.4	0.60±0.06	1.91±0.10	0.72±0.03	1.38±0.11
	0.05	4.9	0.38	1.97	0.59	1.29
Female	0	5.1±0.3	0.51±0.05	1.65±0.09	0.53±0.04	1.70±0.09
	0.01	5.3±0.1	0.49±0.04	1.93±0.04	0.66±0.02	1.87±0.17
	0.05	5.4±0.2	0.72±0.04[a/]	2.09±0.11[a/]	0.67±0.06	1.84±0.04
	0.25	6.6	0.55	1.59	0.67	1.54

Sex	% 2,6-DNT in Feed	Relative Organ Weights (gm/gm brain weight) Liver	Spleen	Kidneys	Heart
Male	0	4.4±0.4	0.30±0.03	1.67±0.21	0.53±0.06
	0.01	3.8±0.6	0.44±0.07	1.40±0.18	0.53±0.06
	0.05	3.8	0.30	1.53	0.46
Female	0	3.0±0.2	0.30±0.01	0.98±0.07	0.31±0.01
	0.01	2.9±0.3	0.27±0.02	1.05±0.08	0.36±0.03
	0.05	2.9±0.2	0.39±0.01[a/]	1.13±0.04	0.36±0.03
	0.25	4.3	0.36	1.03	0.44

Entries are mean ± standard error of two to four mice or single value.

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4/]).

TABLE 75

SUMMARY OF TISSUE LESIONS IN MALE MICE FED 2,6-DNT FOR 4 WEEKS

Lesions[a/]	Dose (% in Feed)															
	0				0.01				0.05				0.25			
Mouse No.:	313	314	315	316	338	339	340	341	363	364	365	366	388	389	390	391
Heart																
Focal epicarditis					1											
Lung																
Broncheogenic carcinoma							+									
Liver																
Focal mononuclear infiltration				1	1	1		1	1	1	1	1	1	1	1	1
Focal necrosis											1					
Extramedullary hematopoiesis															1	
Intestine																
Pinworms	±	+			±				±			+		+	±	
Kidney																
Chronic interstitial nephritis	1	1	1	1		1		1	1	1	1					1
Urinary Bladder																
Focal cystitis						1										
Testis																
Retarded spermatogenesis					1						1					
Atrophy and/or aspermatogenesis													3	3	3	4
Brain																
Focal mononuclear infiltration						1			1					1		
Perivascular cuffing			1		1											
Spleen																
Extramedullary hematopoiesis													1			1
Bone Marrow																
M/E ratio	1.6	1.4	1.6	1.5	1.8	2.4	1.6	2.8	2.1	2.3	2.6	2.3	2.5	1.7	1.9	1.4

Tissues not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

TABLE 76

SUMMARY OF TISSUE LESIONS IN FEMALE MICE FED 2,6-DNT FOR 4 WEEKS

Lesions[a] / Mouse No.:	Dose (% in Feed) 0				0.01				0.05				0.25			
	413	414	415	416	438	439	440	441	463	464	465	466	488	489	390	491
Heart																
Focal myocarditis	1															
Lung																
Chronic murine pneumonia					3					1	±					
Bronchopneumonia					3											
Liver																
Focal mononuclear infiltration	1		1	1		1	2	1	1	1	1	1	2	1	1	1
Focal necrosis			1				1									
Extramedullary hematopoiesis													1	1	1	
Intestine																
Pinworms															±	+
Kidney																
Chronic pyelitis					1											
Chronic interstitial nephritis	1	1	1	1						1	1	1	1	1		
Focal tubular degeneration													1			
Urinary Bladder																
Focal cystitis			1												1	
Ovaries																
Inactive (not cycling)			±			+				+			+	+	+	+
Uterus																
Metritis						1										
Brain																
Focal mononuclear infiltration				1												
Perivascular cuffing			1	1												
Skeletal Muscle																
Focal Myositis							1									
Spleen																
Extramedullary hematopoiesis													1	1	1	
Bone Marrow																
M/E Ratio	1.6	1.8	1.8	1.7	1.9	1.7	2.4	2.0	1.7	1.4	2.5	2.0	2.3	2.4	2.1	1.6

Tissues not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

TABLE 77

SUMMARY OF TISSUE LESIONS IN MALE MICE FED 2,6-DNT FOR 13 WEEKS

Lesions[a/] / Mouse No.:	Dose (% in Feed) 0		0.01				0.05		
	305	306	330	331	332	333	355	356	357
Liver									
Focal mononuclear infiltration	1	1	1	±	1	1	1	1	1
Extramedullary hematopoiesis	1							1	
Bile duct hyperplasia								±	
Intestine									
Pinworms		+					+	+	+
Kidney									
Chronic interstitial nephritis	1	3					±		
Testis									
Retarded spermatogenesis						1			
Brain									
Focal mononuclear infiltration	1	1							
Perivascular cuffing	1								
Spleen									
Extramedullary hematopoiesis	1	1	1	1	1	1	1	1	1
Bone Marrow									
M/E Ratio	1.4	1.3	-	-	-	-	-	-	-

Tissue not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

TABLE 78

SUMMARY OF TISSUE LESIONS IN FEMALE MICE FED 2,6-DNT FOR 13 WEEKS

Lesions[a/] / Mouse No.:	Dose (% in Feed) 0				0.01				0.05				0.25
	405	406	407	408	430	431	432	433	455	456	457	458	480
Liver													
Focal mononuclear infiltration	1	1	1	1	1		1	1	1	+	1	1	2
Extramedullary hematopoiesis										1	1	1	2
Bile duct hyperplasia									2				1
Kidney													
Focal nononuclear infiltration		1								1		+	
Chronic interstitial nephritis									1				
Skeletal Muscle													
Focal myositis													1
Brain													
Focal mononuclear infiltration										1			
Adrenal													
Vacuolation	3	1	3	1								1	
Spleen													
Extramedullary hematopoiesis	1	1				2		1	2	1	1	1	2
Bone Marrow													
M/E Ratio	1.6	1.5	1.6	1.4	-	-	-	-	-	-	-	-	1.5

Tissue not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 - marked; 4 = markedly severe.

TABLE 79

SUMMARY OF TISSUE LESIONS IN MALE MICE FED 2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a/] / House No.:	Dose (% in Feed) 0				0.01				0.25			
	309	310	311	312	334	335	336	337	384	385	386	387
Liver												
Focal mononuclear infiltration		1			1	1		1				2
Extramedullary hematopoiesis	1								1		1	1
Bile duct hyperplasia												1
Kidney												
Chronic interstitial nephritis	1	1	1						1	1	1	2
Brain												
Focal mononuclear infiltration		±	±							1		
Perivascular cuffing	1											
Skeletal Muscle												
Focal mononuclear infiltration	1											
Spleen												
Extramedullary hematopoiesis	2								1		1	
Lymphoid hyperplasia					3	3	2	2				
Bone Marrow												
M/E Ratio	1.7	1.9	1.9	2.2	-	-	-	-	1.3	b/	1.3	b/

Tissue not listed were normal

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

b/ Smear not readable.

TABLE 80

SUMMARY OF TISSUE LESIONS IN FEMALE MICE FED 2,6-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a/]	Dose (% in Feed)															
	0				0.01				0.05				0.25			
Mouse No.:	409	410	411	412	434	435	436	437	459	460	461	462	484	485	486	487
Liver																
Focal mononuclear infiltration		1	1	1		1		1	1	1	1		2		1	1
Extramedullary hematopoiesis														1	1	±
Bile duct hyperplasia														±		
Pancreas																
Focal mononuclear infiltration																1
Kidney																
Focal mononuclear infiltration	±	1														1
Chronic interstitial nephritis													1	1		
Brain																
Focal mononuclear infiltration				±												
Perivascular cuffing																±
Spleen																
Extramedullary hematopoiesis			1						1			2		1	1	1
Lymphoid hyperplasia					3	3	2	1								
Bone Marrow																
M/E Ratio	2.3	1.7	2.4	1.9	-	-	-	-	-	-	-	-	1.2	1.3	1.4	1.3

Tissue not listed were normal

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

TABLE 81

SUMMARY OF TISSUE LESIONS IN MALE MICE FED 2,6-DNT
FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

	Dose (% in Feed)					
	0			0.01		0.05
Lesions[a/] Mouse No.:	301	302	303	326	327	351
Liver						
Focal mononuclear infiltration	1			1		1
Pancreas						
Focal mononuclear infiltration			1			1
Kidney						
Chronic interstitial nephritis		1	1			+
Spleen						
Extramedullary hematopoiesis				1	1	1
Bone Marrow						
M/E Ratio	1.4	1.5	1.5	-	-	-

Tissue not listed were normal

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

TABLE 82

SUMMARY OF TISSUE LESIONS IN FEMALE MICE FED 2,6-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a/] / Mouse No.:	Dose (% in Feed)											
	0				0.01				0.05			0.25
	401	402	403	404	426	427	428	429	451	452	453	476
Liver												
Focal mononuclear infiltration		1	1	1	1	1	1	1	1	1		1
Extramedullary hematopoiesis												1
Pancreas												
Focal mononuclear infiltration			1									
Intestine												
Pinworms										+		
Kidney												
Focal mononuclear infiltration	1			2								1
Chronic interstitial nephritis									1	1	1	
Brain												
Focal mononuclear infiltration				1								±
Perivascular cuffing				1								±
Skeletal Muscle												
Focal mononuclear infiltration												1
Spleen												
Extramedullary hematopoiesis						1	1	1	1	1	1	1
Bone Marrow												
M/E Ratio	1.7	1.6	b/	b/	-	-	-	-	-	-	-	1.3

Tissue not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

b/ Smear not readable.

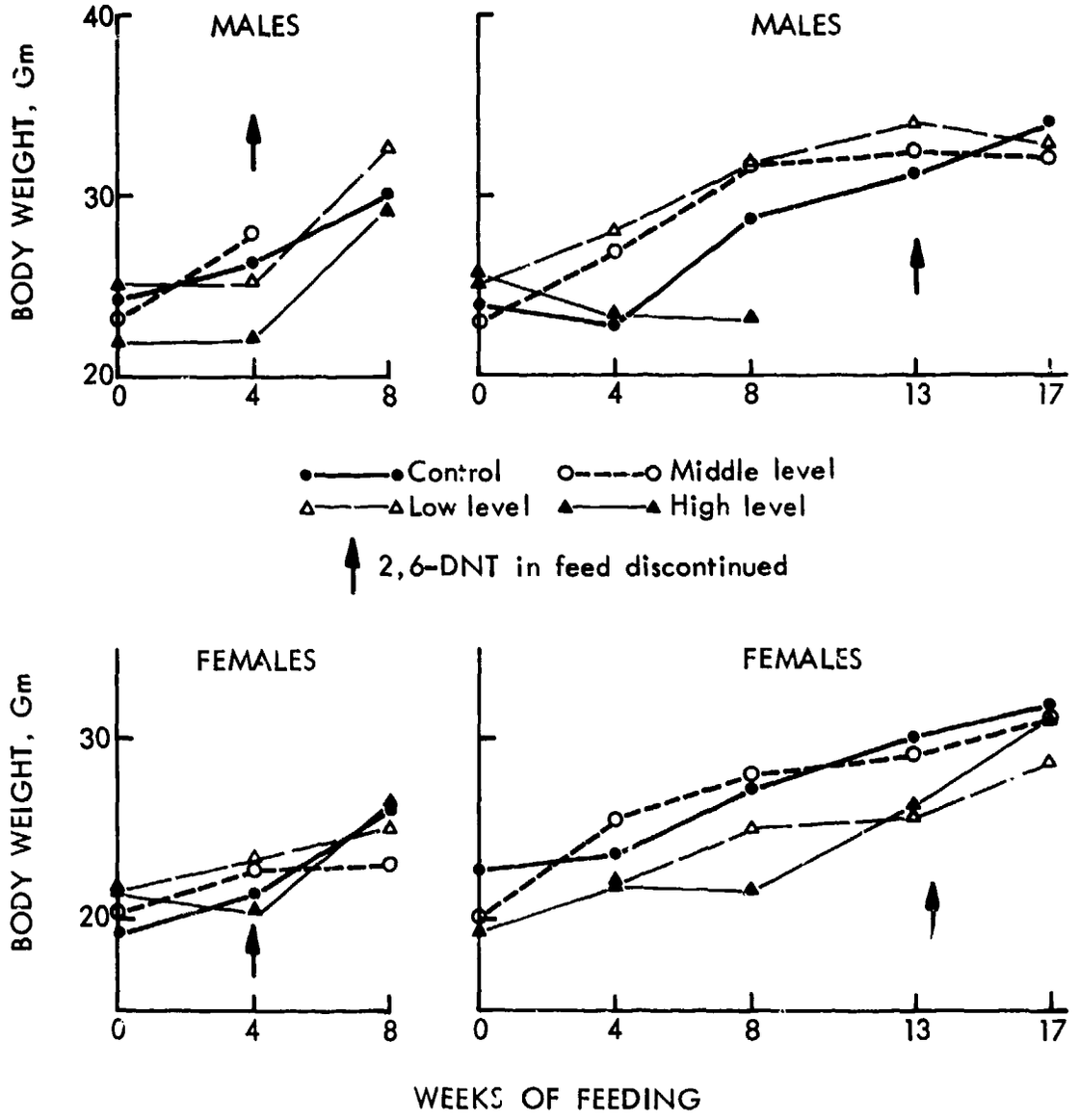

Figure 2 - Body Weights of Mice Fed Various Levels of 2,6-DNT

IV. DISPOSITION AND METABOLISM

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IV. DISPOSITION AND METABOLISM

A. Disposition and Metabolism of 2,6-DNT in Various Species

Absorption, distribution, biotransformation, and excretion of 2,6-DNT were studied in rats.[1] No additional studies have been performed. The present toxicological studies imply that mice absorbed 2,6-DNT as well as rats. It is different for 2,4-DNT which was not well absorbed by mice, and was less toxic to mice than to rats.[6]

B. Biliary Excretion of 2,6-DNT in Rats

The results were discussed in IV.B. of the report on 2,4-DNT,[6] which includes data on all dinitrotoluene isomers and chemically-related compounds.

C. Effect of 2,6-DNT on Drug Metabolizing Enzyme

1. Introduction

The effect of 2,6-DNT on the ability of rats to metabolize test compounds was studied. The test compounds selected for this study were zoxazolamine and nitroanisole. The in vivo metabolism of zoxazolamine was followed by the duration of the loss of the righting reflex following treatment. The in vitro metabolism of nitroanisole in liver was measured in terms of the formation of p-nitrophenol.

2. Material and Methods

Male rats were fed diets that contained 0.25% of 2,6-DNT for 4 weeks, as described in II.A.2. Rats in the positive control group received intraperitoneal injections of 50 mg/kg of sodium phenobarbital twice daily for 3 days. At the end of treatment, the zoxazolamine paralysis time and nitroanisole O-demethylase activity were determined.

Zoxazolamine was administered i.p. to rats at a dose of 45 mg/kg in a vehicle of 0.2 N HCl. The duration of paralysis was measured in terms of the loss of the righting reflex. The values are reported as the mean ± S.E. and the test of significance was the two-sample rank[16] test. The level of significance was selected at $P < 0.05$.

The metabolism of nitroanisole by livers was measured in an in vitro system. Rats were sacrificed by decapitation and the livers were removed, weighed, and homogenized in 4 volumes of 1.15% potassium chloride.

The homogenate was centrifuged at 9,000 x g for 30 minutes. The incubation medium contained 15 µmoles magnesium chloride, 15 µmoles glucose-6-phosphate, 3 µmoles p-nitroanisole, 0.5 ml of the 9,000 x g liver supernatant, and 0.5 ml of 0.5 M sodium phosphate buffer pH 7.8. Reactions were conducted for 20 minutes in a shaking water bath at 37°C. The reaction was terminated by the addition of 0.5 ml of 40% formalin and the color was developed with 0.5 ml of 0.8 N sodium hydroxide. The product formed was measured spectrophotometrically at 420 nm. The relationship of µmoles p-nitrophenol formed = Absorbance Units/10.22 was used to quantitate product formed. The Lowry protein assay[17] was used to measure protein content. The activity was expressed as nanomoles p-nitrophenol/mg protein. The values are reported as the mean ± S.E. The test and level of significance were the same as described above.

3. Results

The results on the zoxazolamine paralysis in rats are summarized in Table 83. Pretreatment with either phenobarbital sodium or a toxic, but not lethal, level of 2,6-DNT in the feed (0.25%) for 4 weeks significantly stimulated the metabolism of zoxazolamine and reduced the duration of paralysis relative to control rats.

The results on nitroanisole O-demethylase activity in rat livers are summarized in Table 84. Again, both pretreatments significantly increased the activity of the enzyme relative to control.

4. Discussion and Conclusion

A toxic, but not lethal, level of 2,6-DNT in the feed (0.25%) for 4 weeks significantly increased the activity of some liver drug-metabolizing enzymes in rats: those which metabolize zoxazolamine and nitroanisole O-demethylase. During the toxicity studies, rats were affected by 2,6-DNT and then adapted while continuing to take the 2,6-DNT. This was most evident in increasing feed consumption and decreasing anemia between week 4 and week 13 in the rats fed 0.25% of 2,6-DNT. If the enzyme activities increased by 2,6-DNT feeding include some enzymes responsible for detoxifying 2,6-DNT, this effect is readily explained. When similar studies were carried out on the isomer 2,4-DNT,[6] no such increase in enzyme activity was observed.

D. Summary

The high level of 2,6-DNT in the feed (0.25%) for 4 weeks decreased zoxazolamine paralysis time and increased hepatic nitroanisole O-demethylase activity in rats, indicating an increase in drug-metabolizing enzyme activity in the liver.

TABLE 83

ZOXAZOLAMINE PARALYSIS IN MALE RATS AFTER TREATMENT WITH 2,6-DNT OR PHENOBARBITAL

Treatment	Duration (min)
Control	111 ± 3 (9)[c]
2,6-DNT(0.25%)[a]	68 ± 6 (9)[d]
Phenobarbital[b]	52 ± 10 (7)[d]

a/ Incorporated into diet and fed for 4 weeks.
b/ 50 mg/kg i.p. twice daily for 3 days.
c/ Mean ± S.E. (number of observations).
d/ Significantly different from control (two sample rank test[16]).

TABLE 84

NITROANISOLE O-DEMETHYLASE ACTIVITY IN LIVERS FROM MALE RATS AFTER TREATMENT WITH PHENOBARBITAL OR 2,6-DNT

Treatment	Activity[c]
Control	3.8 ± 0.3 (4)[d]
2,6-DNT(0.25%)[a]	6.7 ± 0.6 (4)[e]
Phenobarbital[b]	19.0 ± 0.5 (4)[e]

a/ Incorporated into diet and fed for 4 4 weeks.
b/ 50 mg/kg i.p. twice daily for 3 days.
c/ Nmoles ρ-nitrophenol/mg protein.
d/ Mean ± S.E. (number of observations).
e/ Significantly different from control (two sample rank test[16]).

V. GENERAL SUMMARY AND CONCLUSIONS

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V. GENERAL SUMMARY AND CONCLUSIONS

A. Toxic Doses

In dogs, no nontoxic dose was found. However, the lowest dose tested (4 mg/kg/day) had only mild adverse effects in some dogs, so it is probably the minimal toxic dose. Higher doses gave much dose-related toxicity, with 20 mg/kg/day being fatal to some dogs after 9 weeks and 100 mg/kg/day lethal to all within 8 weeks.

Rats were somewhat less sensitive to 2,6-DNT, since 7 mg/kg/day was nontoxic. Larger doses, 35 mg/kg/day in males or 37 mg/kg/day in females and 145 or 155 mg/kg/day, produced a variety of toxicity, with more severe, earlier effects in the high dose rats.

Mice were similar to rats, with 11 mg/kg/day a no-effect dose, and 51 mg/kg/day in males or 55 mg/kg/day in females and 289 mg/kg/day in males or 299 mg/kg/day in females causing dose-related toxicity.

There was considerable individual variation in onset and degree of toxicity.

B. Target Organs

As usual, the nonspecific effects of decreased feed consumption and weight gain were seen in all species.

The blood was severely affected in all species. The primary effect was methemoglobinemia. Numerous sequelae included Heinz bodies, reticulocytosis, anemia, and extramedullary hematopoiesis.

Males of all species had depressed spermatogenesis. In some cases this was so severe that testicular atrophy resulted.

Bile duct hyperplasia, sometimes accompanied by hepatic degeneration or elevated serum enzymes, was seen in all species.

Dogs had two toxic effects not seen in other species. The principal cause of death was incoordination, leading to rigid paralysis. No lesions were seen in the neuromuscular system, so the pathogenesis of this effect is unknown. Renal degeneration and elevated BUN were also seen.

C. Special Studies

2,6-DNT treatment did not increase the serum levels of immunoblobin E in dogs and rats.

Mutagenicity tests in rats gave some positive results, an increase in chromatid breaks in lymphocyte cultures and an increase in tetraploids in kidney cultures. Test with Chinese hamster ovary cultures were negative. The sporadic, weakly positive results may be coincidental.

When the toxic high dose of 2,6-DNT was fed to rats for 4 weeks, there were increases in drug-metabolizing activity, as reflected in decreased zoxazolamine paralysis time and increased hepatic nitroanisole-O-demethylase activity.

D. Research Needed

The results seen here with 2,6-DNT were very similar to those seen in parallel studies with its isomer 2,4-DNT, reported elsewhere. The primary difference was that 2,6-DNT had similar toxic doses in both rodent species tested, while 2,4-DNT is poorly absorbed by, and less toxic to mice. Other differences (less common lesions, mutagenic and drug-metabolizing enzyme effects) were relatively sporadic and inconsistent in comparison with the common effects (the primary target organs, blood, testes, neuromuscular system).

Because of these similarities, and the predominance of 2,4-DNT in usual isomer mixtures, it is probably acceptable to set criteria on the basis of 2,4-DNT effects and evaluate exposures on the basis of total DNT content.

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APPENDIX I

MANUAL FOR

HEMATOLOGY, CLINICAL LABORATORY TESTS, HISTOPATHOLOGY, STATISTICAL ANALYSIS, AND NORMAL VALUES

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January 1977

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HEMATOLOGY, CLINICAL LABORATORY TESTS, HISTOPATHOLOGY, STATISTICAL ANALYSIS, AND NORMAL VALUES

I. HEMATOLOGY AND CLINICAL LABORATORY TESTS

The usual blood sample from dogs is 8 ml, from monkeys 4 ml, and from rats 0.3 ml for hematology and about 8 ml for full analysis at termination.

A. Hematology

The following hematological analyses are performed on all blood samples from rats, dogs and monkeys.

1. Erythrocyte and leukocyte counts: A Coulter Electronic Particle Counter with 100 μ aperture is used.[1] Particle-free diluents (Isoton for RBC, Zap-Oglobin in Isoton for WBC, Coulter Electronics, Inc.) are counted to establish the background. Each blood sample is counted in duplicate. For each test day, two control blood samples (Diagnostic Technology, Inc.) are counted separately in duplicate.

2. Hematocrit: Hematocrit is determined in capillary tubes using a microcapillary centrifuge (International Equipment Company, Model MB). Two control blood samples (Diagnostic Technology, Inc.) are measured separately in duplicate.

3. Hemoglobin: Hemoglobin is measured as cyanomethemoglobin.[2] Each blood sample is measured in duplicate. Cyanomethemoglobin (Coulter Electronics, Inc.) is used as the standard. For each assay, two levels of the standard are used and two control blood samples (Diagnostic Technology, Inc.) are measured in duplicate.

4. Methemoglobin (Met-Hb): Met-Hb is measured by the method of Dubowski.[3] A positive control is made by adding potassium ferricyanide to control blood.

5. Heinz bodies: Heinz bodies are stained with methyl violet and the percent of Heinz bodies is calculated.

6. Mean corpuscular volume (MCV): MCV is calculated as follows:

$$\text{MCV } (\mu^3) = \frac{\text{Hematocrit} \times 10}{\text{Erythrocytes in millions/mm}^3}$$

7. Mean corpuscular hemoglobin (MCHb): MCHb is calculated as follows:

$$\text{MCHb } (\mu\mu g) = \frac{\text{Hemoglobin (gm \%) x 10}}{\text{Erythrocytes in millions/mm}^3}$$

8. Mean corpuscular hemoglobin concentration (MCHbC): MCHbC is calculated as follows:

$$\text{MCHbC (gm \%)} = \frac{\text{Hemoglobin (gm \%) x 100}}{\text{Hematocrit}}$$

9. Differential leukocyte counts: Wright's stain is used to stain the leukocytes for examination.

10. Reticulocyte count: Reticulocytes are counted by the methylene blue method using the Miller disc.[4]

11. Platelet count: A Coulter Electronic Particle Counter with 70 μ aperture is used.[5] Particle-free Isoton is used as diluent and counted to establish the background. At weekly intervals, platelets are also visually counted in a hemocytometer with a phase microscope for comparison.[6]

12. Clotting time (dog and monkey): Clotting time is determined by the capillary tube procedure using two capillary tubes.[7] The time elapsed from the appearance of the blood from the animal and coagulation in either tube is measured.

B. Clinical Blood Tests

The following clinical blood chemistry tests are performed on all blood samples from dogs and monkeys and on blood samples from rats at termination.

1. Blood glucose: Fasting blood glucose is determined by Stein's hexokinase method.[8] Standard glucose solution (Dade) is used to establish a standard curve. For each assay, one level of the standard and two controls (Reference Serum, Worthington; and Validate, General Diagnostics) are measured.

2. Serum glutamic-oxaloacetic transaminase (SGOT): SGOT is measured by the method of Amador and Wacker.[9] Validate and Reference Serum are used as the enzyme reference for each assay.

3. Serum glutamic-pyruvic transaminase (SGPT): SGPT is measured by the method of Henry et al.[10] Validate and Reference Serum are used as the enzyme reference for each assay.

4. Alkaline phosphatase: Alkaline phosphatase is measured by the method of Bowers and McComb.[11] Validate and Reference Serum are used as the enzyme reference for each assay.

5. BUN: BUN is measured using the BUN Strate Kit (General Diagnostic) which is based on the urease method.[12] Three levels of Calibrate (General Diagnostics) are used to establish a standard curve. For each assay, two controls (Calibrate I and Validate) are used as the reference.

6. Creatinine: Creatinine is measured by a modified kinetic alkaline picrate procedure.[13] Creatinine Standard Solutions (Sigma Chemical Company) are used to establish a standard curve. For each assay, two levels of the standard and two controls (Calibrate I and Validate) are used as reference.

7. Lactate dehydrogenase (LDH): LDH is measured by the method of Wacker et al.[14] Precinorm E and Precipath E (Boehringer, Mannheim Corporation) are used as the enzyme controls for each assay.

8. α-Hydroxybutyrate dehydrogenase (α-HBDH): α HBDH is measured by the method of Rosalki and Wilkinson.[15] Precinorm E and Precipath E are used as the enzyme controls for each assay.

9. Creatine phosphokinase (CPK): CPK is measured by the improved procedure of Rosalki[16] based on the methods of Oliver.[17] Precinorm E and Precipath E are used as the enzyme controls for each assay.

C. Urinalysis

Urine samples are collected from animals before and during treatment as are the blood samples. The urine from rats is collected by slight manipulation of their body, and samples within each group are pooled. The monkeys and dogs are placed individually in metabolism cages, and urine is collected in the stainless steel pan. The urine from each dog and the pooled urine from rats are tested and examined for the following:

1. Protein: Urinary protein is determined with Labstix (Ames Company, Elkhart, Indiana).

2. Sugar: Urinary glucose and reducing substance are determined with Labstix (Ames Company).

3. Microscopic examination: Urine samples are centrifuged and the supernatant discarded. The residue is resuspended and examined microscopically for the presence of erythrocytes, leukocytes, epithelial cells, and crystals under high power field and for casts under low power field.

A positive urine control prepared with known amounts of protein and glucose in saline adjusted to pH 6.0 is run with each assay to check the reliability of the Labstix.

D. Occult Blood in Feces

Fecal samples are collected from animals before and during treatment as are the blood and urine samples. Occult blood in the feces is determined with Hematest Reagent Tablets (Ames Company, Elkhart, Indiana). A positive control (whole blood) and a negative control (distilled water) are included with each assay to check the reliability of the Hematest tablets.

E. Precision of Hematology and Clinical Blood Chemistry Tests

1. Reproducibility

For erythrocyte and leukocyte counts, hematocrit, hemoglobin, and the various clinical blood chemistry tests, the same control blood samples or control standards are used for day-to-day assays. The replication of results are excellent and are summarized in Table A.

The determination of differential leukocyte counts and reticulocyte counts are performed by experienced personnel. At weekly intervals, a blood sample is counted by two or more personnel to confirm the accuracy of the counting. Also at weekly intervals, the platelet counts obtained from a Coulter Electronic Particle Counter are compared with the direct visual counts in a hemocytometer using a phase microscope.

2. Reproducibility Within a Test Day

At monthly intervals, a blood sample is taken from a control dog and six or more determinations for erythrocyte, leukocyte, reticulocyte, and platelet counts, hemoglobin, and various clinical blood chemistry tests are performed to establish the reproducibility within an assay. The results are summarized in Table B.

3. Proficiency Test Service

We subscribe to the Proficiency Test Service of the Institute for Clinical Science, Hahnemann Medical College, Philadelphia, Pennsylvania (F. Wm. Sunderman, M.D., Director). On the first day of each month, this service sends two samples containing two different sera or solutions to all subscribers for measurements of one or more of the parameters usually analyzed in clinical laboratories. Participants report their results on a form furnished by the service. On the 15th day of the month, each participant receives a report from the service which includes: the results of a statistical analysis of the values reported by all the participating laboratories; a current review of pertinent methodology; a comprehensive bibliography; and validation of the results which the participating laboratory reported. This service enables each participating laboratory to obtain an unbiased and critical assessment of its proficiency in relation to that of 1,000 or so other clinical laboratories throughout the country. The service has been in continuous operation since 1949 and was given endorsement by the American Society of Clinical Pathologists in 1952 and by the Association of Clinical Scientists in 1957 and 1968. Our results have been found to be satisfactory and are summarized in Table C.

II. HISTOPATHOLOGY

A. Necropsy and Gross Examination

At termination or prior to imminent death, rats are killed with ether, and dogs and monkeys with an overdose of sodium pentobarbital. Animals that die on tests are kept refrigerated but not frozen until necropsy. The general physical condition and nutritional status of each animal at the time of death or termination are observed and recorded. Necropsy is performed as soon as possible after death. Gross changes of all tissues are carefully examined and recorded.

B. Organ Weights

The brain, liver, spleen, kidneys, adrenals, thyroids and gonads are trimmed free from surrounding tissues and weighed. The organ weight to body weight and/or brain weight ratios are then calculated.

C. Tissues for Microscopic Examination

Tissues to be examined include the eye, skin (breast), trachea, lung, tongue (except rat), salivary gland, liver, gallbladder (except rats), pancreas, esophagus, fundic and pyloric stomach, duodenum, jejunum, ileum, cecum, colon, kidneys, urinary bladder, gonads, and accessory organs, diaphragm and gracilis muscle, anterior pituitary, thyroids/parathyroids, adrenals, tonsil (except rat), thymus, spleen, prescapular (except rats) and mesenteric lymph nodes, rib bone with bone marrow, brain (sagittal section for rats; coronal sections of cerebral cortex, cerebellum, and brain stem for dog and monkey), spinal cord (lumbosacral plexus, dog and monkey), sciatic nerve and any other structures not mentioned which show abnormal gross changes.

D. Fixation and Staining of Tissues

All tissues are cut not to exceed 1 cm in thickness for fixation. For most tissues, neutral buffered 10% formalin is used. Sufficient volume of fixing solution is used and the tissues are changed to a fresh solution after 24 hours. The fixed tissues are processed in an Autotechnicon for dehydration, clearing, and infiltration and then embedded in paraffin. Routine H & E staining is used to stain the sectioned tissues for microscopic examination.

Supplementary tissue fixatives and staining techniques may be employed for more positive identification of special lesions such as calcification, pigments, fat deposition and other abnormal changes.

III. STATISTICAL ANALYSIS

Data are analyzed statistically using the Dunnett's multiple comparison procedure following an analysis of variance,[18] or our modification of this procedure for uneven numbers among groups. The chosen criterion significance is $p < 0.05$. The means of each group at various intervals during treatment are compared with pretreatment levels. For most experiments in beagles, three baseline (pretreatment) levels are obtained. The baseline levels for each animal are averaged and the mean is used in the analysis. In addition, the means of the various treated groups are compared with that of the control group at the respective time intervals.

IV. NORMAL VALUES

A. Hematology, Clinical Laboratory Tests and Bone Marrow

Since June 1971, we have used about 180 rhesus monkeys (Woodard Research Corporation, Herndon, Virginia, Primate Imports, Port Washington, New York, and PrimeLabs, Inc., Farmingdale, New Jersey) for various studies. The peripheral blood elements and clinical blood chemistry values of these monkeys before treatment and the myeloid/erythroid (M/E) ratio of the bone marrow of the monkeys used as normal controls varied among individual animals. The mean ± S.D. and the range of the various parameters for the males and females are summarized in Tables D and E, respectively.

Since September 1971, we have used about 525, 5 to 9 months old, beagles dogs (AKC registered, Hazelton Research Animals, Inc.). The peripheral blood elements, clinical blood chemistry values and the M/E ratio of the bone marrow varied considerably among individual dogs. The mean ± S.D. and the ranges of the various parameters for the males and females are summarized in Tables H and I, respectively.

During the same period, we have used about 500, 7 to 10 weeks old, male albino rats (CD® Strain, Charles River Breeding Laboratories). As for the dogs, the individual variations of the peripheral blood elements, clinical blood chemistry values and the M/E ratio of the bone marrow were large. The mean ± S.D. and the ranges of the various parameters for these male rats are summarized in Table L.

B. Absolute and Relative Organ Weights

Organ weights, both absolute and relative to body weight, of rhesus monkeys, beagle dogs, and albino rats are summarized in Tables F and G, J and K, and M, respectively. These were control animals used between June 1971 and December 1976.

C. Presence of Various Substances in the Urine

Various substances occasionally occurred in the urine of monkeys, dogs and rats. The results are summarized in Table N. Large percentage of urine samples from monkeys contained epithelial cells, i.e., 34.7% to 52.0%. Other substances occurred in 8.1% or less of the urine samples.

In dogs, protein, erythrocytes, leukocytes and epithelial cells were present in 19.1 to 21.6%, 16.5 to 19.8%, 22.6 to 24.6% or 24.7 to 25.7%, respectively, of the samples from dogs collected for analysis. Glucose,

crystals, and casts occurred in less than 2% of these samples. Some dogs had been bled and returned to the metabolism cages before the urine was removed for analysis. The high incidence of some of these substances in the urine of these dogs might be due to contamination with the fecal material and traces of blood dropped in the cage. Special care to avoid contamination has been undertaken.

In rats, large percentage of urine samples contained protein, i.e., 29.8 to 36.0%. A few samples contained erythrocytes, leukocytes, epithelial cells and crystals.

D. Occult Blood in the Feces

Less than 10% of the feces samples from monkeys or dogs was positive with the Hematest for occult blood. The results are summarized in Table O.

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TABLE A

REPRODUCIBILITY AMONG TEST DAYS ON THE SAME CONTROL SAMPLES OR STANDARDS[a/]

	No. of Determinations	Mean ± S.D.	Range
Erythrocytes (x $10^6/mm^3$)			
Normal level	20	4.51 ± 0.07	4.36 - 4.67
Abnormal level	20	2.32 ± 0.04	2.25 - 2.40
Hematocrit (vol %)			
Normal level	20	44.3 ± 0.40	44 - 45
Abnormal level	20	22.8 ± 0.60	22 - 24
Hemoglobin (gm %)			
Normal level	20	14.2 ± 0.20	13.6 - 14.5
Abnormal level	20	7.4 ± 0.20	6.9 - 7.8
Leukocyte Counts (x $10^3/mm^3$)			
Normal level	20	7.3 ± 0.50	6.8 - 8.7
Abnormal level	20	17.6 ± 0.80	16.3 - 18.7
Fasting Blood Glucose (mg %)	20	163.0 ± 7.5	151 - 178
SGOT (IU/ℓ)	23	61.7 ± 3.9	55 - 68
SGPT (IU/ℓ)	23	51.3 ± 2.6	46 - 55
Creatinine (mg %)	18	2.2 ± 0.3	1.6 - 2.6
BUN (mg %)	19	9.8 ± 0.2	9.5 - 10.2
Bilirubin (mg %)	11	0.8 ± 0.1	0.8 - 1.0
Alkaline Phosphatase (IU/ℓ)	22	71.6 ± 5.4	62 - 80
CPK	11	153.0 ± 7.7	139 - 161
LDH	8	98.0 ± 2.4	95 - 101
HBDH	8	226.0 ± 7.2	214 - 238

a/ Performed in December 1976.

TABLE B

REPRODUCIBILITY WITHIN A TEST DAY
ON THE SAME SPECIMEN[a/]

	Mean ± S.D.[b/]	Range
Erythrocytes (x $10^6/mm^3$)	5.90 ± 0.14	5.73 - 6.08
Reticulocytes (%)	0.63 ± 0.12	0.44 - 0.79
Hematocrit (vol %)	46.8 ± 0.6	46.0 - 47.5
Hemoglobin (gm %)	16.1 ± 0.2	15.8 - 16.1
Platelets (x $10^5/mm^3$)	1.56 ± 0.07	1.49 - 1.66
Leukocytes (x $10^3/mm^3$)	10.8 ± 0.4	10.2 - 11.3
Bands (%)	0 ± 0	0 - 0
Neutrophils (%)	64.3 ± 3.1	61 - 69
Lymphocytes (%)	29.0 ± 4.9	23 - 35
Eosinophils (%)	3.2 ± 0.8	2 - 4
Basophils (%)	0 ± 0	0 - 0
Monocytes (%)	3.4 ± 0.9	3 - 5
Atypical (%)	0 ± 0	0 - 0
Nucleated RBC (%)	0 ± 0	0 - 0
Methemoglobin (gm %)	0 ± 0	0 - 0
Fasting Glucose (mg %)	96.7 ± 3.0	32 - 101
SGOT (IU/ℓ)	23.2 ± 2.8	21 - 28
SGPT (IU/ℓ)	25.3 ± 2.1	24 - 28
Creatinine (mg %)	0.6 ± 0.1	0.5 - 0.6
BUN (mg %)	9.0 ± 0.0	9 - 9
Alkaline Phosphatase (IU/ℓ)	63.5 ± 1.1	62 - 65
CPK	44.0 ± 1.6	43 - 46
LDH	38.5 ± 1.6	37 - 40
HBDH	42.0 ± 1.6	40 - 43

a/ Performed in October 1976.
b/ Six determinations from an adult beagle blood sample.

TABLE C

PROFICIENCY TEST SERVICE (PTS) REPORTS (1975-1976)[a/]

Unknowns	MRI Results	PTS Results	Participating Laboratories (10-90 Percentiles) Median	Participating Laboratories (10-90 Percentiles) Mean	Acceptable Performance[b/]
Hemoglobin	13.8 gm %	13.8	13.8	13.8	13.6 - 14.0
	18.1 gm %	17.9	17.9	17.8	17.6 - 18.2
Serum Protein	6.6 mg %	7.1	7.0	7.0	6.7 - 7.3
Fasting Glucose	272.0 mg %	264.5	266.0	263.0	240 - 290
	229.0 mg %	221.4	220.5	222.5	200 - 240
BUN	12.1 mg %	12.0	12.0	12.2	11.0 - 13.0
	38.4 mg %	40.1	40.3	39.2	36.0 - 44.0
Creatinine	1.0 mg %	1.0	1.0	1.0	0.8 - 1.3
	4.3 mg %	4.4	4.5	4.4	3.9 - 4.9
Bilirubin	3.9 mg %	4.16	4.15	4.14	3.5 - 4.6
	1.3 mg %	1.78	1.80	1.77	1.5 - 2.1
Cholesterol	175.0 mg %	161.4	161.0	162.0	145 - 175
	100.0 mg %	109.8	109.4	111.0	98 - 120
Ca	15.7 meq/ℓ	15.4	15.4	15.3	14.1 - 16.4
	9.5 meq/ℓ	9.8	9.8	9.8	9.2 - 10.3
Na	156.0 meq/ℓ	155.8	156.0	155.5	153 - 158
K	7.3 meq/ℓ	7.5	7.5	7.5	7.3 - 7.7
Cl	96.0 meq/ℓ	97.8	98.0	97.5	96 - 101
	78.0 meq/ℓ	79.4	79.0	80.0	77 - 83
Mg	1.0 meq/ℓ	1.1	1.1	1.2	0.9 - 1.4
	1.9 meq/ℓ	2.0	2.0	2.1	1.8 - 2.3

a/ To date, we have received unknowns for phosphorus, uric acid, and serum iron. We do not routinely perform these determinations.

b/ Based on values submitted by participants by 10th of month.

TABLE D

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF MALE RHESUS MONKEYS[a/]

	Male Rhesus Monkeys			
	Number Studied	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x $10^6/mm^3$)	108	3.74 ± 0.50	5.51 ± 0.45	3.75 - 6.61
Reticulocytes (%)	108	3.74 ± 0.50	0.97 ± 0.82	0.07 - 2.41
Hematocrit (vol %)	108	3.74 ± 0.50	43.0 ± 2.6	37.0 - 50.0
Hemoglobin (gm %)	108	3.74 ± 0.50	13.4 ± 0.8	10.8 - 15.4
MCV (μ^3)	108	3.74 ± 0.50	77.8 ± 7.0	69.6 - 117.3
MCHb ($\mu\mu g$)	108	3.74 ± 0.50	24.4 ± 1.8	22.0 - 33.6
MCHbC (mg %)	108	3.74 ± 0.50	31.4 ± 1.3	27.2 - 34.1
Platelets (x $10^5/mm^3$)	99	3.76 ± 0.50	3.08 ± 0.45	0.80 - 7.10
Leukocytes (x $10^3/mm^3$)	108	3.74 ± 0.50	10.4 ± 4.9	3.8 - 30.1
Neutrophils I (%)	108	3.74 ± 0.50	0.18 ± 0.45	0 - 2
Neutrophils M (%)	108	3.74 ± 0.50	39.30 ± 17.72	10 - 83
Lymphocytes (%)	108	3.74 ± 0.50	56.83 ± 17.74	13 - 84
Eosinophils (%)	108	3.74 ± 0.50	1.91 ± 2.42	0 - 13
Monophils (%)	108	3.74 ± 0.50	1.37 ± 1.58	0 - 7
Basophils (%)	108	3.74 ± 0.50	0.04 ± 0.20	0 - 2
Atypical cells (%)	108	3.74 ± 0.50	0.00 ± 0.00	0 - 0
Nucleated RBC (%)		3.74 ± 0.50	0.00 ± 0.00	0 - 0
Fasting Glucose (mg %)	100	3.76 ± 0.51	96.9 ± 15.2	59 - 127
SGOT (IU/ℓ)	100	3.76 ± 0.51	33.7 ± 9.2	20 - 60
SGPT (IU/ℓ)	100	3.76 ± 0.51	31.3 ± 7.8	15 - 46
Alkaline Phosphatase (IU/ℓ)	100	3.76 ± 0.51	360.0 ± 116.0	143 - 501
BUN (mg %)	100	3.76 ± 0.51	19.5 ± 7.5	12 - 65
Proth. Time (sec)	62	3.91 ± 0.44	10.2 ± 0.7	9.3 - 11.9
Serum Creat. (mg %)	100	3.76 ± 0.51	1.1 ± 0.3	0.6 - 1.8
Bilirubin				
Total (mg %)	62	3.91 ± 0.44	0.1 ± 0.2	0.0 - 0.8
Direct (mg %)	62	3.91 ± 0.44	0.0 ± 0.0	0.0 - 0.0
BSP 15 min (% ret.)	62	3.91 ± 0.44	18.0 ± 7.4	2 - 34
Na (mEq/ℓ)	62	3.91 ± 0.44	154.0 ± 19.1	144 - 179
K (mEq/ℓ)	62	3.91 ± 0.44	4.8 ± 0.6	3.9 - 5.7
Cl (mEq/ℓ)	62	3.91 ± 0.44	109.0 ± 6.4	93 - 118
Ca (mEq/ℓ)	62	3.91 ± 0.44	5.2 ± 0.4	4.2 - 6.3
Mg (mEq/ℓ)	62	3.91 ± 0.44	1.6 ± 0.1	1.2 - 1.8
Bone Marrow				
Myeloid/erythroid ratio	15	3.65 ± 0.41	1.5 ± 0.3	1.5 - 2.1

a/ Data collected between June 1971 and December 1976.

TABLE E

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF FEMALE RHESUS MONKEYS[a/]

	Female Rhesus Monkeys			
	Number Studied	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	81	3.51 ± 0.48	5.33 ± 0.40	4.25 - 6.03
Reticulocytes (%)	81	3.51 ± 0.48	1.07 ± 0.54	0.35 - 3.31
Hematocrit (vol %)	81	3.51 ± 0.48	41.5 ± 2.8	30.0 - 46.0
Hemoglobin (gm %)	81	3.51 ± 0.48	13.1 ± 1.0	7.9 - 14.1
MCV (μ^3)	81	3.51 ± 0.48	77.7 ± 5.3	66.5 - 95.2
MCHb ($\mu\mu$g)	81	3.51 ± 0.48	24.6 ± 1.7	17.6 - 29.7
MCHbC (mg %)	81	3.51 ± 0.48	31.6 ± 1.4	26.6 - 34.2
Platelets (x 10^5/mm^3)	81	3.51 ± 0.48	3.11 ± 1.23	1.85 - 7.90
Leukocytes (x 10^3/mm^3)	81	3.51 ± 0.48	9.5 ± 3.9	3.2 - 24.8
Neutrophils I (%)	81	3.51 ± 0.48	0.10 ± 0.43	0 - 3
Neutrophils M (%)	81	3.51 ± 0.48	36.41 ± 13.32	13 - 56
Lymphocytes (%)	81	3.51 ± 0.48	60.38 ± 13.26	41 - 79
Eosinophils (%)	81	3.51 ± 0.48	2.28 ± 3.10	0 - 18
Monophils (%)	81	3.51 ± 0.48	0.75 ± 0.98	0 - 4
Basophils (%)	81	3.51 ± 0.48	0.05 ± 0.22	0 - 1
Atypical cells (%)	81	3.51 ± 0.48	0.00 ± 0.00	0 - 0
Nucleated RBC (%)	74	3.56 ± 0.50	0.00 ± 0.00	0 - 0
Fasting Glucose (mg %)	81	3.51 ± 0.48	92.1 ± 15.3	57 - 116
SGOT (IU/ℓ)	81	3.51 ± 0.48	32.1 ± 7.6	20 - 70
SGPT (IU/ℓ)	81	3.51 ± 0.48	30.1 ± 7.6	12 - 39
Alkaline Phosphatase (IU/ℓ)	81	3.51 ± 0.48	349.9 ± 112.3	148 - 572
BUN (mg %)	81	3.51 ± 0.48	17.3 ± 4.2	13 - 29
Proth. Time (sec)	59	3.56 ± 0.43	10.5 ± 0.9	9.7 - 12.3
Serum Creat. (mg %)	81	3.51 ± 0.48	1.1 ± 0.3	0.6 - 1.7
Bilirubin				
Total (mg %)	81	3.51 ± 0.48	0.1 ± 0.1	0.0 - 0.8
Direct (mg %)	81	3.51 ± 0.48	0.0 ± 0.0	0.0 - 0.0
BSP 15 min (% ret.)	59	3.56 ± 0.43	16.4 ± 8.3	5 - 34
Na (mEq/ℓ)	59	3.56 ± 0.43	158.2 ± 6.5	147 - 174
K (mEq/ℓ)	59	3.56 ± 0.43	4.8 ± 0.7	3.9 - 6.2
Cl (mEq/ℓ)	59	3.56 ± 0.43	109.0 ± 6.1	95 - 113
Ca (mEq/ℓ)	59	3.56 ± 0.43	5.3 ± 0.5	4.3 - 6.3
Mg (mEq/ℓ)	59	3.56 ± 0.43	1.6 ± 0.2	1.3 - 2.0
Bone Marrow				
Myeloid/erythroid ratio	11	3.49 ± 0.62	1.4 ± 0.3	1.0 - 1.8

a/ Data collected between June 1971 and December 1976.

TABLE F

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MALE RHESUS MONKEYS[a/]

Organ Weight	Absolute Mean ± S.D.	Absolute Range
Liver (gm)	82 ± 17	64 - 122
Spleen (gm)	4.6 ± 1.8	2.0 - 9.3
Kidneys (gm)	15.1 ± 3.8	8.0 - 22.0
Adrenals (gm)	0.73 ± 0.15	0.45 - 0.86
Thyroids (gm)	0.57 ± 1.30	0.37 - 0.81
Testes (gm)	1.29 ± 0.67	0.53 - 3.30
	Relative (per kg body weight)	
	Mean ± S.D.	**Range**
Liver (gm)	23.4 ± 2.5	18.8 - 30.4
Spleen (gm)	1.25 ± 0.47	0.57 - 2.38
Kidneys (gm)	4.13 ± 0.92	2.20 - 6.43
Adrenals (mg)	201 ± 44	129 - 254
Thyroids (mg)	154 ± 42	86 - 250
Testes (gm)	0.34 ± 0.11	0.18 - 0.53

a/ Data collected between September 1971 and December 1976 from 17 monkeys weighing 3.71 ± 0.48 kg, used as control animals.

TABLE G

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF FEMALE RHESUS MONKEYS[a/]

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	83 ± 17	64 - 122
Spleen (gm)	3.8 ± 1.4	2.0 - 6.0
Kidneys (gm)	14.5 ± 2.8	11.0 - 20.0
Adrenals (gm)	0.68 ± 0.16	0.53 - 1.14
Thyroids (gm)	0.60 ± 0.20	0.37 - 1.11
Ovaries (gm)	0.28 ± 0.10	0.14 - 0.45
	Relative (per kg body weight)	
	Mean ± S.D.	**Range**
Liver (gm)	25.4 ± 5.8	19.2 - 37.4
Spleen (gm)	1.16 ± 0.49	0.60 - 1.89
Kidneys (gm)	4.40 ± 0.86	3.20 - 6.25
Adrenals (mg)	212 ± 80	138 - 438
Thyroids (mg)	173 ± 66	97 - 346
Ovaries (mg)	82 ± 28	43 - 140

a/ Data collected between September 1971 and December 1976 from 11 monkeys weighing 3.39 ± 0.58 kg, used as controls.

TABLE H

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF MALE BEAGLE DOGS[a/]

	Male Beagle Dogs				
	Number Studied	Age (months)	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	276	4 - 7	8.3 ± 1.7	5.55 ± 0.73	3.62 - 7.60
Reticulocytes (%)	284	4 - 7	8.3 ± 1.7	0.72 ± 0.46	0.04 - 4.35
Hematocrit (vol %)	276	4 - 7	8.3 ± 1.7	41.6 ± 3.5	31 - 50
Hemoglobin (gm %)	276	4 - 7	8.3 ± 1.7	13.5 ± 1.4	10.0 - 16.9
MCV (μ^3)	276	4 - 7	8.3 ± 1.7	75.6 ± 8.3	56.7 - 127.1
MCHb ($\mu\mu$g)	276	4 - 7	8.3 ± 1.7	24.6 ± 3.0	17.1 - 41.7
MCHbC (mg %)	276	4 - 7	8.3 ± 1.7	32.5 ± 1.5	28.1 - 40.3
Platelets (x 10^5/mm^3)	270	4 - 7	8.4 ± 1.7	2.91 ± 1.02	0.93 - 6.35
Leukocytes (x 10^3/mm^3)	284	4 - 7	8.3 ± 1.7	11.9 ± 3.5	4.6 - 24.6
Neutrophils I (%)	284	4 - 7	8.3 ± 1.7	0.55 ± 1.06	0 - 6
Neutrophils M (%)	284	4 - 7	8.3 ± 1.7	56.81 ± 9.47	22 - 80
Lymphocytes (%)	284	4 - 7	8.3 ± 1.7	37.94 ± 9.26	13 - 71
Eosinophils (%)	284	4 - 7	8.3 ± 1.7	2.76 ± 2.93	0 - 16
Monophils (%)	284	4 - 7	8.3 ± 1.7	1.78 ± 1.84	0 - 11
Basophils (%)	284	4 - 7	8.3 ± 1.7	0.01 ± 0.10	0 - 2
Atypical cells (%)	284	4 - 7	8.3 ± 1.7	0.11 ± 0.37	0 - 2
Nucleated RBC (%)	284	4 - 7	8.3 ± 1.7	0.02 ± 0.10	0 - 2
Fasting Glucose (mg %)	284	4 - 7	8.3 ± 1.7	100.9 ± 12.6	66 - 134
SGOT (IU/ℓ)	276	4 - 7	8.3 ± 1.7	23.2 ± 7.4	11 - 59
SGPT (IU/ℓ)	276	4 - 7	8.3 ± 1.7	25.7 ± 7.9	8 - 46
Alkaline Phosphatase (IU/ℓ)	276	4 - 7	8.3 ± 1.7	73.3 ± 18.5	21 - 133
BUN (mg %)	284	4 - 7	8.3 ± 1.7	12.1 ± 3.3	4 - 23
Bone Marrow					
Myeloid/erythroid ratio	34	5 - 9	9.4 ± 1.6	1.6 ± 0.4	1.1 - 3.0

a/ Data collected between September 1971 and December 1976.

TABLE I

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF FEMALE BEAGLE DOGS[a/]

	Female Beagle Dogs				
	Number Studied	Age (months)	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x $10^6/mm^3$)	257	4 - 7	6.9 ± 1.3	5.59 ± 0.73	3.27 - 7.75
Reticulocytes (%)	265	4 - 7	6.9 ± 1.3	0.74 ± 0.52	0.04 - 5.05
Hematocrit (vol %)	257	4 - 7	6.9 ± 1.3	42.3 ± 3.5	32 - 51
Hemoglobin (gm %)	257	4 - 7	6.9 ± 1.3	13.7 ± 1.3	11.0 - 18.6
MCV (μ^3)	257	4 - 7	6.9 ± 1.3	76.7 ± 9.7	55.8 - 128.4
MCHb ($\mu\mu g$)	257	4 - 7	6.9 ± 1.3	24.8 ± 3.3	17.1 - 41.6
MCHbC (mg %)	257	4 - 7	6.9 ± 1.3	32.3 ± 1.6	28.7 - 40.4
Platelets (x $10^5/mm^3$)	227	4 - 7	6.9 ± 1.3	3.08 ± 1.15	1.08 - 7.95
Leukocytes (x $10^3/mm^3$)	265	4 - 7	6.9 ± 1.3	10.9 ± 3.4	3.8 - 26.9
Neutrophils I (%)	265	4 - 7	6.9 ± 1.3	0.54 ± 1.16	0 - 7
Neutrophils M (%)	265	4 - 7	6.9 ± 1.3	57.08 ± 10.10	31 - 85
Lymphocytes (%)	265	4 - 7	6.9 ± 1.3	37.15 ± 10.46	10 - 61
Eosinophils (%)	265	4 - 7	6.9 ± 1.3	2.37 ± 2.25	0 - 13
Monophils (%)	265	4 - 7	6.9 ± 1.3	1.94 ± 2.01	0 - 9
Basophils (%)	265	4 - 7	6.9 ± 1.3	0.01 ± 0.09	0 - 1
Atypical cells (%)	265	4 - 7	6.9 ± 1.3	0.11 ± 0.43	0 - 4
Nucleated RBC (%)	265	4 - 7	6.9 ± 1.3	0.03 ± 0.17	0 - 2
Fasting Glucose (mg %)	248	4 - 7	6.9 ± 1.3	99.6 ± 14.4	55 - 130
SGOT (IU/ℓ)	257	4 - 7	6.9 ± 1.3	23.5 ± 7.2	6 - 52
SGPT (IU/ℓ)	257	4 - 7	6.9 ± 1.3	25.3 ± 7.0	8 - 49
Alkaline Phosphatase (IU/ℓ)	257	4 - 7	6.9 ± 1.3	73.5 ± 19.2	30 - 146
BUN (mg %)	265	4 - 7	6.9 ± 1.3	12.4 ± 3.3	4 - 26
Bone Marrow					
Myeloid/erythroid ratio	34	5 - 9	7.8 ± 1.4	1.4 ± 0.3	1.1 - 2.4

a/ Data collected between September 1971 and December 1976.

TABLE J

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MALE BEAGLE DOGS[a/]

Organ Weight	Absolute Mean ± S.D.	Absolute Range
Liver (gm)	264 ± 51	166 - 384
Spleen (gm)	58 ± 25	22 - 167
Kidneys (gm)	53 ± 10	32 - 71
Adrenals (gm)	1.12 ± 0.26	0.74 - 1.75
Thyroids (gm)	1.03 ± 0.32	0.55 - 2.50
Testes (gm)	6.60 ± 4.56	1.32 - 18.00
	Relative (per kg body weight)	
	Mean ± S.D.	**Range**
Liver (gm)	27.9 ± 4.2	19.6 - 42.3
Spleen (gm)	6.0 ± 2.0	2.8 - 12.5
Kidneys (gm)	5.6 ± 0.8	4.0 - 7.7
Adrenals (mg)	117 ± 25	70 - 165
Thyroids (mg)	108 ± 34	56 - 211
Testes (gm)	0.67 ± 0.39	0.13 - 1.67

a/ Data collected between September 1971 and December 1976 from 51 dogs, weighing 9.3 ± 1.8 kg, used as control animals.

TABLE K

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF FEMALE BEAGLE DOGS[a/]

Organ Weight	Absolute Mean ± S.D.	Absolute Range
Liver (gm)	218 ± 51	106 - 322
Spleen (gm)	48 ± 21	16 - 103
[illegible] (gm)	43 ± 9	24 - 71
[illegible]nals (gm)	1.04 ± 0.26	0.49 - 1.65
[illegible]roids (gm)	0.88 ± 0.25	0.55 - 1.91
Ovaries (gm)	0.74 ± 0.24	0.38 - 1.27
	Relative (per kg body weight)	
	Mean ± S.D.	**Range**
Liver (gm)	28.2 ± 5.0	20.7 - 38.8
Spleen (gm)	6.0 ± 2.3	3.1 - 10.9
Kidneys (gm)	5.5 ± 0.9	3.7 - 7.9
Adrenals (mg)	135 ± 35	67 - 215
Thyroids (mg)	112 ± 31	75 - 219
Ovaries (mg)	96 ± 33	54 - 222

a/ Data collected between September 1971 and December 1976 from 49 dogs, weighing 7.7 ± 1.5 kg, used as control animals.

TABLE L

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF MALE ALBINO RATS[a/]

	Male Rats			Observed Results	
	Number Studied	Age (weeks)	Body Weight (gm) Mean ± S.D.	Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	527	5 - 7	168 ± 22	5.84 ± 0.54	3.24 - 7.60
Reticulocytes (%)	461	5 - 7		3.04 ± 1.80	0.30 - 6.83
Hematocrit (vol %)	525	5 - 7	168 ± 22	45.1 ± 3.2	40 - 58
Hemoglobin (gm %)	525	5 - 7	168 ± 22	13.7 ± 0.9	11.8 - 17.1
MCV (μ^3)	525	5 - 7	168 ± 22	78.1 ± 16.3	62.3 - 104.6
MCHb ($\mu\mu$g)	525	5 - 7	168 ± 22	23.7 ± 2.6	19.2 - 41.0
MCHbC (mg %)	525	5 - 7	168 ± 22	30.5 ± 1.8	21.1 - 36.9
Platelets (x 10^5/mm^3)	473	5 - 7	164 ± 24	4.93 ± 1.23	2.30 - 7.95
Leukocytes (x 10^3/mm^3)	448	5 - 7	164 ± 24	15.4 ± 4.0	6.3 - 20.8
Neutrophils I (%)	448	5 - 7	164 ± 24	0.07 ± 0.31	0 - 3
Neutrophils M (%)	448	5 - 7	164 ± 24	14.1 ± 6.2	4 - 29
Lymphocytes (%)	448	5 - 7	164 ± 24	83.63 ± 6.75	52 - 96
Eosinophils (%)	448	5 - 7	164 ± 24	0.64 ± 0.91	0 - 6
Monophils (%)	448	5 - 7	164 ± 24	1.23 ± 1.73	0 - 13
Basophils (%)	448	5 - 7	164 ± 24	0.01 ± 0.15	0 - 2
Atypical cells (%)	448	5 - 7	164 ± 24	0.01 ± 0.12	0 - 2
Nucleated RBC (%)	448	5 - 7	164 ± 24	0.10 ± 0.42	0 - 4
Fasting Glucose (mg %)	125	10 - 12	348 ± 72	130.9 ± 17.2	94 - 165
SGOT (IU/ℓ)	125	10 - 12	348 ± 72	108.2 ± 34.5	63 - 223
SGPT (IU/ℓ)	125	10 - 12	348 ± 72	34.2 ± 16.5	17 - 120
Alkaline Phosphatase (IU/ℓ)	125	10 - 12	348 ± 72	94.9 ± 30.0	32 - 153
BUN (mg %)	125	10 - 12	348 ± 72	16.4 ± 4.7	8 - 41
Bone Marrow					
Myeloid/erythroid ratio	109	10 - 12	349 ± 63	1.7 ± 0.5	1.0 - 2.6

a/ Data collected between September 1971 and December 1976.

TABLE M

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MALE ALBINO RATS[a/]

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	10.89 ± 2.87	7.18 - 15.09
Spleen (gm)	0.65 ± 0.11	0.34 - 0.89
Kidneys (gm)	2.64 ± 0.37	1.84 - 3.58
Adrenals (mg)	63.6 ± 9.5	21.9 - 73.5
Thyroids (mg)	26.3 ± 5.8	14.3 - 37.7
Testes (gm)	2.98 ± 0.51	1.76 - 3.81
	Relative (per 100 gm body weight)	
	Mean ± S.D.	**Range**
Liver (gm)	2.96 ± 0.42	2.09 - 4.01
Spleen (gm)	0.19 ± 0.08	0.10 - 0.30
Kidneys (gm)	0.76 ± 0.10	0.22 - 0.88
Adrenals (mg)	18.6 ± 5.8	5.8 - 22.4
Thyroids (mg)	7.6 ± 2.7	4.2 - 12.7
Testes (gm)	0.87 ± 0.15	0.23 - 1.09

a/ Data collected between September 1971 and December 1976 from 139 rats, weighing 352 ± 59 gm, used as control animals.

TABLE N

PRESENCE OF VARIOUS SUBSTANCES IN THE URINE OF MALE AND FEMALE MONKEYS, DOGS AND MALE RATS

Species:		Monkeys		Dogs		Rats[a]	
No. of Animals:		141[b]	18	615[b]	112	84[b]	18
No. of Collections:		141	98[c]	615	565[c]	84	56[d]
Glucose:	< 250 mg %	0[e]	2.0 (2)	0.2 (1)	0.7 (4)	0	0
	> 250 mg %	0	0	0.5 (3)	0.2 (1)	0	0
Protein:	< 100 mg %	3.5 (5)	6.1 (6)	19.3 (119)	17.3 (98)	29.8 (25)	36.0 (18)
	> 100 mg %	0	2.0 (2)	2.3 (14)	1.8 (10)	0	0
RBC:[f]	Moderate	1.4 (2)	3.1 (3)	16.4 (101)	13.3 (75)	3.6 (3)	8.0 (4)
	Excessive	0	0	3.4 (21)	3.2 (18)	0	0
WBC:[f]	Moderate	1.4 (2)	2.0 (2)	18.7 (115)	20.9 (118)	0	4.0 (2)
	Excessive	0	0	3.9 (24)	3.7 (21)	0	0
Epithelium:[g]	Moderate	31.2 (44)	44.9 (44)	21.0 (129)	21.9 (124)	0	8.0 (4)
	Excessive	3.5 (5)	7.1 (7)	4.7 (29)	2.8 (16)	0	0
Crystal:[h]	Moderate	0.7 (1)	2.0 (2)	0.2 (1)	0.7 (4)	0	2.0 (1)
	Excessive	0	0	0.2 (1)	0.7 (4)	0	2.0 (1)
Casts:	Positive	0.7 (1)	5.1 (5)	0	0.9 (5)	0	0

a/ Pooled sample of 4-20 rats.
b/ Baseline data collected from all animals employed between September 1971 and December 1976.
c/ Data collected at weekly intervals for 4-7 collections from controls employed between September 1971 and December 1976.
d/ Data collected at 2-week intervals for 2-4 collections from control rats employed between September 1971 and December 1976.
e/ Percent of total (number of samples).
f/ Normal, 10 or less cells; moderate, 10-100 cells; excessive, > 100 cells/field (x 440).
g/ Normal, 5 or less cells; moderate, 5-25 cells; excessive, > 25 cells/field (x 100).
h/ Normal, none; moderate, 1-5 crystals; excessive, > 5 crystals/field (x 100).

TABLE O

PRESENCE OF OCCULT BLOOD IN THE FECES OF MALE AND FEMALE MONKEYS AND DOGS

Species:	Monkeys		Dogs	
No. of Animals:	44[a]	8	118[a]	30
No. of Collections:	44	48[b]	118	156[b]
Occult Blood: Negative	90.9 (40)[c]	95.8 (46)	94.1 (111)	91.7 (143)
Positive	9.1 (4)	4.2 (2)	5.9 (7)	8.3 (13)

a/ Baseline data collected from all animals employed between July 1974 and December 1976.

b/ Data collected at weekly intervals for 4-7 collections from controls employed between July 1974 and December 1976.

c/ Percent of total (number of samples).